INDIAN PHYTOPATHOLOGY

VOLUME XI 1958 *NUMBER 2*

PUBLISHED FOR

INDIAN PHYTOPATHOLOGICAL SOCIETY
I.A.R.I. BUILDING, NEW DELHI-12
INDIA

TABLE OF CONTENTS

MUTATION IN *COLLETOTRICHUM FALCATUM* WENT, THE CAUSAL ORGANISM OF RED ROT OF SUGARCANE. I. INDUCED BY BETA RADIATION

R. S. Vasudeva, M. R. S. Iyengar, B. S. Bajaj and M. S. Chatrath

(Accepted for publication September 1, 1958)

It has been considered that radioactive phosphorus may be a good mutagenic agent in experimentation with living organisms as according to Comar (1955) it gets easily incorporated in the nucleic acids and nucleoproteins of the chromosome and thus chances of hitting the chromosomes with beta particles are sufficiently high. In addition, there will probably be enough energy produced to break the chemical bonds. It is also pointed out that S^{32} is produced out of the incorporated P^{32}, with the result that there may be some molecular rearrangement at the point of decay. It was, therefore, considered interesting to study the effect of various levels of activity of P^{32} on *Colletotrichum falcatum*, the causal organism of red rot of sugarcane. For the purposes of this experimentation strain 244 of *C. falcatum* of this laboratory which is highly virulent and has maintained its virulence for nearly 10 years on artificial culture media and also widely used for varietal resistance tests all over India, was selected. Details about this culture are available in literature (Chona, 1954).

In earlier experiments spores of the fungus were treated either on paper discs or in suspension with different levels of a neutralised aqueous solution of carrier-free radioactive phosphorus obtained from Atomic Energy Research Establishment, Harwell, Berks, (England). In the case of paper discs impregnated with P^{32}, the spores were incubated first at 18°C for 48 hours and then transferred to Richards' medium*. At total levels ranging from 0.75 to 4.5μc there were no effects on the fungus in the liquid medium. However, when the cultures were transferred back to oat meal agar** blackening of the mycelium unlike the controls, was observed in the treated material. On continued subculturing this was found to be only a temporary change and the organism reverted to its original characters.

In the second method a small volume, usually not more than 0.2 ml. of heavy, washed spore - suspension was pipetted into the P^{32} solution (2 ml.) and the suspension was stored at about 7°C. The activity of this final suspension was about 20μc per ml. Controls were also treated similarly except that in place of radioactive phosphorus, sterile distilled water was used. At varying intervals ranging up to 245.0 hours small aliquots of the suspension were removed, diluted and washed several times with sterile water after centrifugation taking aseptic precautions. The washed spores were taken in a small volume of distilled water and uniform aliquots were pipetted into Richards' medium and Dextrose-Asparagine-Thiamine medium*** in flasks. The cultures were incubated at about

*		**		***	
KNO_3	10.0 gms.	Quaker Oats	40 gms.	Dextrose	30.0 gms.
$MgSO_4$	2.5 gms.	Agar Agar	20 gms.	Asparagine	1.0 gm.
KH_2PO_4	5.0 gms.	Distilled water	1000 ml	$MgSO_4\,7H_2O$	0.5 gm.
Sucrose	50.0 gms.			KH_2PO_4	1.5 gms.
$FeCl_3$	Trace			Thiamine	1.0 μg/ml
Distilled water	1000 ml.			Water	1000 ml.

Note:—20 ml. of the liquid medium was used in each 100 ml. flask.

24°C. for three weeks during which the cultures were examined carefully for any changes in growth and morphological characteristics. At this level of radioactivity used (20μc per ml.) again no changes were observed. However, when the spores were treated by a similar method with an activity level of about 170.0μc per ml. for a maximum period of 34 days there was some reduction in the percentage of germination on different substrata (Table I).

TABLE I. Effect of P^{32} (170μc per ml.) on the germination of *Colletotrichum falcatum* spores when treated for 34 days.

Substrate used	Treated spores	Untreated spores
1. Distilled water	10.0%	21.8%
2. Sucrose 1%	2.6%	19.0%
3. Yeast extract 0.2%	11.3%	27.5%

Mass cultures made on oat meal agar from both the treated and untreated spores had the same characters except in one culture where the pink spore masses were thinner than in the controls. When this culture was grown in petriplates marked sectoring was observed. The sectors were markedly black in colour in contrast to the surrounding areas with pink spore masses. Between the sectors there were rays showing thinner and finer spore masses than in the rest of the plate where sporulation was normal. In the sectors themselves the mycelium had a cottony character; the mycelium as well as the substrate was dark coloured (Plate I, Fig. 1). Single spore as well as mass cultures were made on oat meal agar from the areas showing different characters. In the first generation slight differences in the nature of sporulation persisted in the treated series, but on further sub-culturing the cultures reverted to their normal characteristics.

In a variation of the above experiment radioactive phosphorus was incorporated in the Dextrose-Asparagine – Thiamine medium and spores inoculated as previously. The final activity of this suspension was about 240.0μc per ml. The fungus was allowed to grow in the medium at a temperature of 19–23°C. for twenty nine days until good sporulation was observed in both the treated and control series. The spores were collected, washed with sterile water and plated out at a sufficiently high dilution so as to get distinct colonies. The colonies which developed showed differences in regard to the nature of sporulation and texture and pigmentation of the mycelium. At the point of coalescence of most of the colonies accumulation of dark coloured mycelium was observed. Slight cottony growth was also observed at the juncture of colonies, in the similarly plated controls but the mycelial growth was not so extensive as in the treated series (Plate I, Fig. 2). Transfers were made, in each case, from different parts of the colonies as well as the coalescing areas. Cultures obtained from the treated material showed considerable variation in their morphological characteristics. They could be broadly grouped into three categories: (1) Typical, dark, mycelial growth with no apparent pink spore masses as in the well-known dark non-virulent strains of *C. falcatum*, (2) Cultures with abundant, pink spore masses

which were thinner than those in the normal culture and also showing some yellow pigmentation on the agar surface and (3) cultures with abundant thick pink spore masses more or less as in the controls. Some intergrading forms as regards sporulation, mycelial characters, and pigmentation were also observed. In the untreated material a mycelial type apparently similar to Type 1 of the treated series and heavy-sporing type similar to the normal culture were obtained. The heavy sporing types obtained both in the treated and the control series were found to be similar microscopically. In Type 1 of the treated series as well as in the mycelial type obtained from untreated material fairly good sporulation was observed under the microscope, but the nature of sporulation was different in each case. In the mycelial type from the treated series the spores were characteristically smaller in size and of varying shape. Most of the spores did not have the falcate shape, so characteristic of this fungus (Plate II, Fig. 1). The size of the spores varied 9.0 – 21.6 x 4.4 – 7.2μ (mostly 14.4 – 18.0 x 5.4 – 6.3μ) as compared to 21.6 – 28.8 x 4.4 – 5.4μ (mostly 23.4 – 27.0 x 4.5 – 5.4μ) in the control. As this was an interesting variation, a number of single spore as well as mass cultures were made and carried through fifteen more generations. In both the cases the change observed in the spores remained constant. In the mycelial type derived from the untreated material the spores remained normal. The induced variation observed in strain 244 of *C. falcatum* in the present work represents, in our opinion, a stable mutant obtained with the help of radioactive phosphorus.

Summary

A stable mutant of *Colletotrichum falcatum* Went has been obtained with the help of radioactive phosphorus when the fungus was grown on liquid medium containing P^{32} with an initial activity level of about 240μc/ml. The mutant resembles the well-known dark type of *C. falcatum* with lesser sporulation. The spores of the mutant are characteristically smaller in size and vary in shape as compared to the normal falcate shape.

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Comar, C. L. (1955). Radioisotopes in Biology and Agriculture-Principles and Practice. McGraw-hill Book Company, Inc. New York.

PLATE I

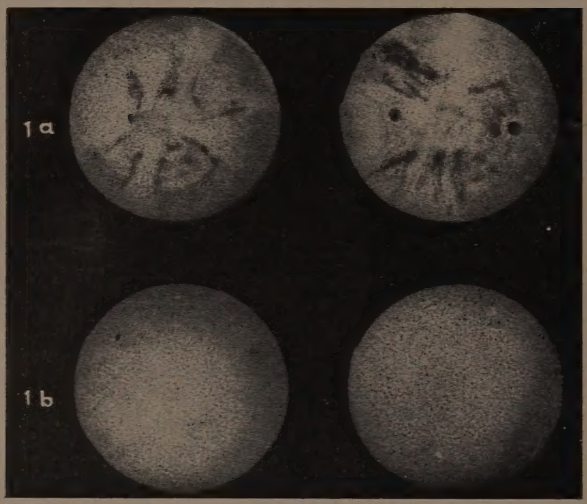


Fig. 1 (a) Temporary changes (sector formation) induced in *C. falcatum* with the aid of P^{32} at an initial activity level of 170μc/ml.

(b) Control plates with uniform sporulation.

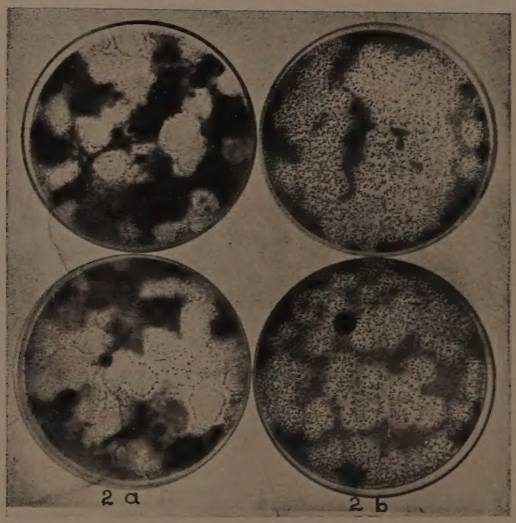


Fig. 2 (a) Colonies with different characters formed out of the treated spores (240 μc/ml. activity).

(b) Uniform sporultion in the control plates.

PLATE II

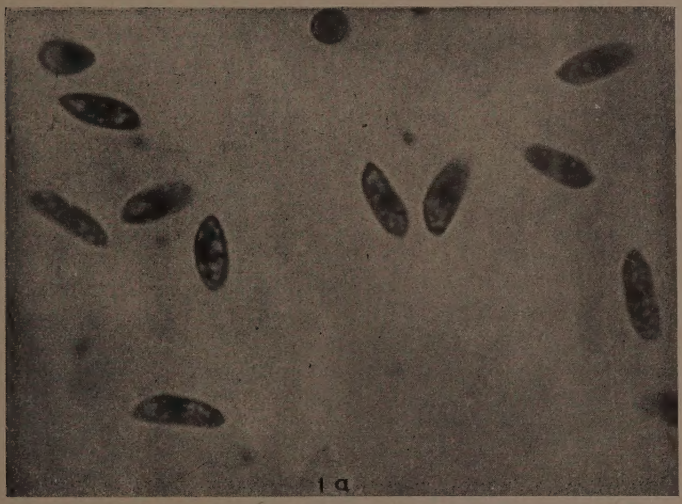
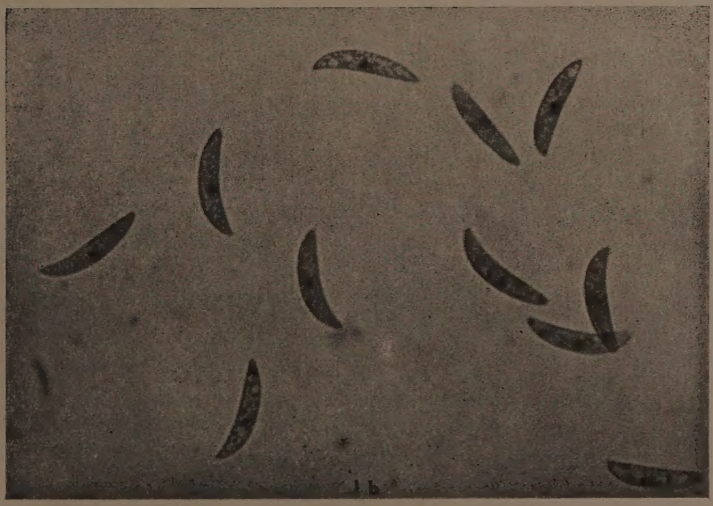


Fig. 1. (a) Spores of the mutant, smaller in size and variable in shape.



(b) Falcate spores of the parent culture.

THE MYXOMYCETES OF THE MUSSOORIE HILLS - XI

K. S. Thind and P. S. Rehill

(Accepted for publication September 5, 1958)

This paper deals with nine more species of Myxomycetes collected from Mussoorie Hills (a few from Dehra Dun) in the North West Himalayas. Three of these *Physarum leucophaeum* Fries, *P. rigidum*, (G. Lister) G. Lister and *Didymium leoninum* Berk. & Br. are new records for India. The first ten contributions (listed under references) give accounts of 61 known species (including one variety), and 4 new species and 1 new form.

The classification of Martin, 1949, has been followed throughout this study, although monographs of Lister and Lister, 1925, and Macbride and Martin, 1934, were freely consulted.

The numbers of the species are serial numbers of the myxomycetous flora of the Mussoorie Hills.

The type collections have been deposited in the Herbarium of the Panjab University and Herbarium Crypt. Ind. Orient. New Delhi. Duplicate material is also deposited in the Botany Department in the State University of Iowa, U.S.A.

The authors are deeply indebted to Dr. G. W. Martin of the State University of Iowa for help in the determination of species and Prof. P. N. Mehra, Head of the Panjab University Botany Department, for providing facilities and encouragement. They are also thankful to Mr. B. Khanna for making illustrations of the fruit bodies.

68. *Physarum nicaraguense* Macbr.

Fructifications sporangiate: sporangia 0.76 – 1.1 mm. wide and 0.5 – 0.76 mm. long, individual lobes 0.2 – 0.3 mm. wide, gregarious, often connate, stipitate, white or ashen white, erect, or nodding, multilobed, lobes 3 – 20 (or even more), or compound-contorted, obconic below, sometimes simple to sparsely lobed: stipe up to 2.5 mm. long and up to 0.75 mm. wide at the base, rarely connate, slender, brown above, dark brown below, lighter coloured above, darker below, longitudinally grooved or fluted, tapering upward: hypothallus distinct, small, dark brown to black, rotate, ridged or reticulate: peridium single, thick, whitish to ashen coloured, covered over with nodular or squamulose lime deposit: dehiscence irregular, peridium rupturing above but persisting below.

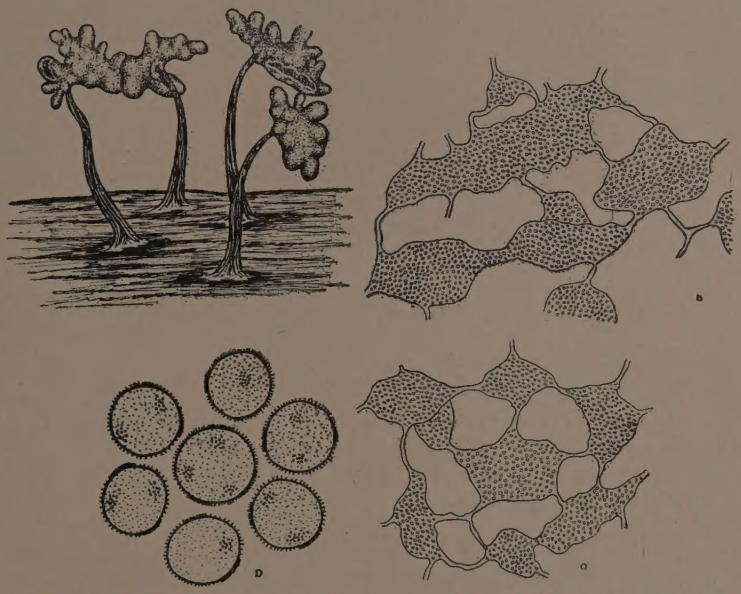
Columella absent, but pseudocolumella formed.

Capillitium abundant, composed of a network of nodes and internodes: nodes, very large, irregular in shape and size, white, calcareous,

often massed together in the centre to form a pseudocolumella, interconnected directly or by internodes: internodes slender, short, hyaline, noncalcareous, scanty.

Spores 10 – 12μ in diameter, black in a mass, violet brown to deep violet under the microscope, globose to subglobose, distinctly and profusely verrucose, often marked by clusters of darker and thicker warts.

Text-Fig. 1, A-D.



Text-Fig. 1. *Physarum nicaraguense* Macbr. A. Prominently lobed and long stipilate sporangia (n. 20), X 20. B. Capillitium (n. 21), X 320. C. Capillitium (n. 20), X 320. D. Prominently verrucose spores with a few small clusters of darker and bigger warts (n. 21), X 1150.

Collected on dead leaves of *Agave* sp., Doiwala, Dehra Dun, August 9, 1954, **263**. On bark and wood, Kansrao, Dehra Dun, August 13, 1953, **264**. On bark of a tree, Saharanpur Road, Dehra Dun, August 4, 1954, **265**.

Collections n. 263 and n. 264 are typical of *P. nicaraguense* Macbr. However, collection n. 265 is atypical in having many simple to sparsely lobed (2–3 lobed) sporangia with a complete transition between them. Its simple sporagnia are globose-obovate, slightly laterally compressed, transversely elongated, frequently connate and 0.3–0.5 mm. in diameter, 0.37–0.5 x 0.5–0.67 mm. when transversely elongated. Such a sporangial

type does not seem to have been reported for this species before. However, it is not felt that these differences observed are beyond the range of a single species.

69. *Physarum leucophaeum* Fries.

Fructifications sporangiate: sporangia 0.26 – 0.57 mm. in diameter, gregarious, stipitate, erect, white to ashen in colour, globose to subglobose; stipe 0.1 – 1.14 mm. long and up to 50 μ wide at the base, erect, dark brown to almost black below and pale brown above, tapering upwards, usually smooth: hypothallus small, concolorous with the base of the stipe, rotate: peridium single, thin, memberanous, gray, transparent, covered over with lime deposits or flakes which have irregular processes and which sometimes join with the neighbouring lime deposits, thus the lime deposits sometimes give an appearance of irregular reticulations, lime crystals granular and rounded: dehiscence irregular, the peridium rupturing irregularly at the top, while the lower portion remaining persistent in the form of petaloid lobes.

Columella absent.

Capillitium abundant, consists of a net work of nodes and internodes: nodes white, calcareous, small, angular in shape, few, united to each other by internodes which are more prominently and abundantly developed than the nodes: internodes long, slender, hyaline, non-calcareous and branched to form a net-work.

Spores 9 – 11 μ in diameter, black in a mass, violet under the microscope, globose to subglobose, profusely and distinctly verrucose, warts also present in the form of darker and prominent clusters.

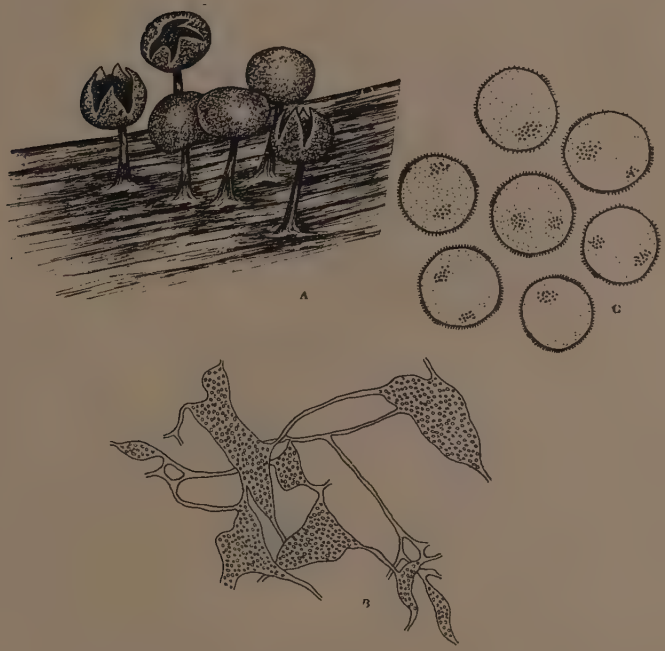
Text-Fig. 2, A–C. (see page 99)

Collected on dead leaves and stems of sugarcane and a grass, also on wild animals' refuse, Doiwala, Dehra Dun, August 9, 1953, **266**. New record in India.

This Dehra Dun collection closely resembles *P. leucophaeum* Fries but differs from it in the lack of bluish tinge of sporangia, lack of rugose character of its stipe and in the possession of clusters of darker and thicker warts on its spores. These slight differences are considered here as mere variations within the scope of the species.

70. *Physarum tenerum* Rex.

Fructifications sporangiate: sporangia 0.4 – 0.6 mm. in diameter, gregarious, stipitate, nodding, yellow or turmeric coloured, globose: stipe 1.75 – 2.5 mm. long and up to 0.15 mm. wide at the base, long, dark orange at the base and pale yellow towards the top, slightly calcareous, rugose, thick below, gradually tapering upwards: hypothallus minute, dark brown, membranous, rotate: peridium single, thin, membranous, hyaline, trans-



Text-Fig. 2. *Physarum leuophaeum* Fries, A. Fructifications with some sporangia forming petaloid lobes during dehiscence, X 20. B. Capillitium, X 320. C. Verrucose spores showing a few clusters of darker and bigger warts, X 1150.

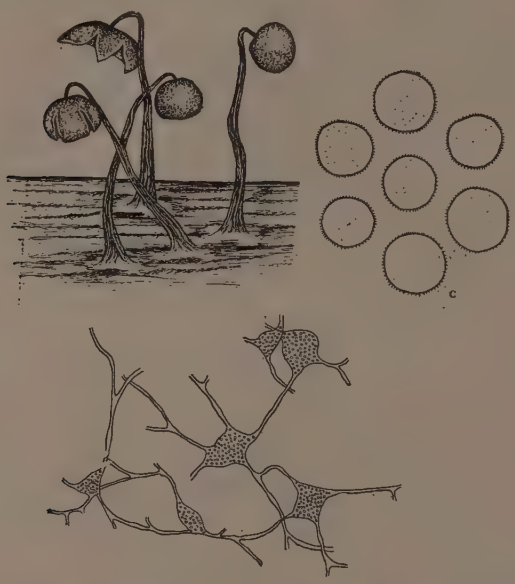
parent, thickly covered with rounded deep yellow, calcareous flakes: dehiscence petaloid, the sporangia rupture longitudinally into irregular, petal-like lobes, which become reflexed and remain persistent.

Columella absent.

Capillitium abundant, composed of a network of nodes and internodes: nodes few, small, mostly spindle-shaped or fusiform, sometimes rounded, calcareous, brown to dark brown in colour, inter-connected with slender, violaceous, long, branched and non-calcareous inter nodes.

Spores 8 – 10.5 μ in diameter, mostly 9 μ in diameter, dark brown to black in a mass, violet under the microscope, globose to subglobose, profusely verrucose, warts minute and showing a tendency to arrange in clusters in some spores.

Text-Fig. 3, A–C.



Text-Fig. 3. *Physarum tenerum* Rex, A. Fructifications with 2 sporangia forming petaloid lobes during dehiscence, spores showing small clusters of darker warts in a few spores, X 1150.

Collected on dead wood, Chakrata Toll, Mussoorie, September 2, 1953, **267**.

This mussoorie fungus undoubtedly belongs to *P. tenerum* Rex and is characterized by bright yellow sporangia, dehiscing by petal-like lobes,, opaque, slightly calcareous stipes, small brown nodes, and minutely warted spores, mostly 9 μ in diameter.

71. *Physarum rigidum* (G. Lister) G. Lister.

Fructifications sporangiate: sporangia 0.5 – 1 mm. in diameter, gregarious, mostly nodding, stipitate, dull yellow or olive-yellow, iridescent where devoid of yellowish lime deposits, depressed globose or lenticular, umbilicate above, frequently connate: stipe 1 – 1.5 mm. long, up to 40 μ wide at the base, long, nodding at the top, brown, lighter above and darker below, noncalcareous, longitudinally ridged, tapering upward: hypothallus small, concolorous with the base of the stipe, rotate, ridged irregularly, noncalcareous: peridium single, thin, membranous, dull yellow, calcareous, calcareous matter in the form of yellowish, scattered lime deposits: dehiscence irregular, usually by splitting of the peridium from the top into petaloid lobes which remain persistent at the base.

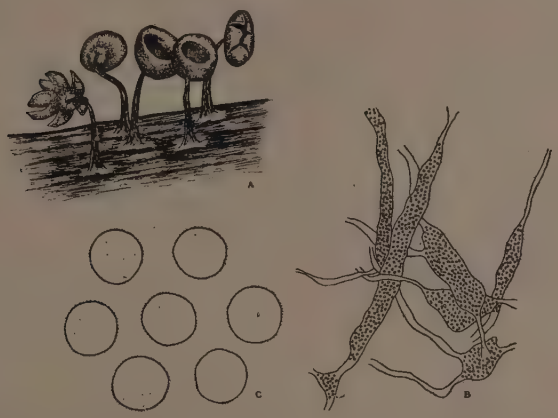
Columella absent.

Capillitium abundant, springing from the peridial floor, capillitium threads mostly vertical and composed of abundant nodes and internodes: nodes yellow, fusiform to elongated, calcareous: internodes hyaline, relatively thicker and rigid, noncalcareous, sparingly branched.

Spores 8 – 9.6 μ in diameter, black in a mass, violet-brown under the microscope, globose to subglobose, inconspicuously verrucose, warts also present in clusters.

Plasmodium dirty yellow.

Text-Fig. 4, A–C.



Text-Fig. 4. *Physarum rigidum* (G. Lister) G. Lister, A. Depressed globose (lenticular) sporangia umbilicate above, with persistant petaloid lobes observed in one of them after dehiscence, X 20. B. Capillitium with fusiform to elongated nodes, X320. C. Minutely verrucose spores, also containing small clusters of darker and thicker warts, X1150.

Collected on rotting wood and *Aporpium* sp. growing on the same piece of wood, The Park, Mussoorie, July 28, 1954, **268**. New record in India.

The spores of the Mussoorie collection are smaller for the species. Capillitium consisting almost entirely of slender rod-like tubes enclosing lime granules was not observed in this collection.

72. *Craterium aureum* (Schum.) Rost.

Fructifications sporangiate: sporangia 0.3 – 0.5 x 0.2 – 0.38 mm., gregarious, sometimes scattered, stipitate, yellow, erect, oblong to ovoid, apex rounded: stipe 0.25 – 0.57 mm. in height, up to 0.08 mm. wide, rugose, yellow, concolorous or somewhat deeper coloured than the sporan-

gium, calcareous, lime crystals yellow, rounded and granular: hypothallus distinct but minute, rotate, cream coloured to yellow, calcareous: peridium single, thin above, thick below, encrusted with yellow, granular, rounded lime crystals: dehiscence irregular, the peridium rupturing at the upper thin portion irregularly, or sometimes by a lid-like portion, while the lower thicker portion persisting like a small cup with irregular margin.

Columella absent, but a pseudocolumella may be formed.

Capillitium well developed and abundant, consisting of nodes and internodes: nodes yellow, small, irregular in shape and size, calcareous, often massed together in the centre so as to form a pseudocolumella: internodes slender to somewhat thick, hyaline, branched and non-calcareous.

Spores 8 – 9.6 μ in diameter, black in a mass, violaceous brown under the microscope, globose to subglobose, strongly and profusely verrucose.

Text-Fig. 5, A–D.

Text-Fig. 5. *Craterium aureum* (Schum.) Rost. A. Fructifications, X20. B. Dehisced sporangia with capillitium exposed, X50. C. Capillitium with pseudocolumella X 200. D. Prominently verrucose spores, X 1150.

Collected on dead and decaying leaves of *Saccharum* sp. and *Dalbergia sissoo* Roxb., Nalapani, Dehra Dun, August 3, 1953, **269**. On dead leaves of *Quercus incana*, *Berberis* sp., and other plants, on dead twigs, and on leaves of an alive grass, Chakrata Toll, Mussoorie, July 21, 1954, **270**.

Both of these collections (n. 269 and n. 270) undoubtedly belong to *Craterium aureum* (Schum.) Rost. and are characterized by yellow, sti-

pitate sporangia dehiscing irregularly leaving the persistent lower portion with an uneven margin, dense yellow nodes often massed together into a pseudocolumella, and profusely verrucose spores, 8 – 9.6 μ in diameter.

The species was recorded from Landour, Mussoorie, by Lodhi, 1934. However, his description is very incomplete as the amount of material available to him was very small.

73. *Physarella oblonga* (Berk. & Curt.) Morgan.

Fructifications sporangiate: sporangia 0.4 – 1.25 mm. long and 0.3 – 0.75 mm. wide, gregarious or scattered, stipitate, erect, or nodding, grayish yellow, sometimes gray, cylindrical or oblong, deeply umbilicate at the top to cup shaped or sub-infundibuliform; stipe 1–3 mm. long and up to 0.37 mm. wide at the base, erect or bending and nodding above, reddish brown, longitudinally rugose, tapering upward: hypothallus distinct, reddish brown, rotate, venose: peridium single, thick, firm, yellowish gray, or gray, flecked with yellow calcareous scales (sometimes gray), introverted at the top so as to give a bell shaped appearance to the sporangium, bearing on the inner surface of the exterior walls stout yellow spines which penetrate to the interior walls: dehiscence irregular, peridium rupturing at the top in a lobate fashion, the petal-like lobes soon becoming reflexed and thus exposing the yellow spiny processes (trabecules) on its interior wall, and leaving the cylindrical inner wall protruding as a yellow and tubular pseudocolumella also flecked with yellow scales, trabeculae up to 0.2 x 0.02 mm. The stipe is extended within the sporangium to meet the tubular pseudocolumella.

Capillitium abundant, composed of slender, hyaline to violaceous, sparingly branching and anastomosing threads and bearing a few small, fusiform, yellow sometimes whitish, calcareous nodes or nodular expansions. Capillitial threads radiate out from the inner wall but supported by stout yellow spines running parallel to the capillitial threads.

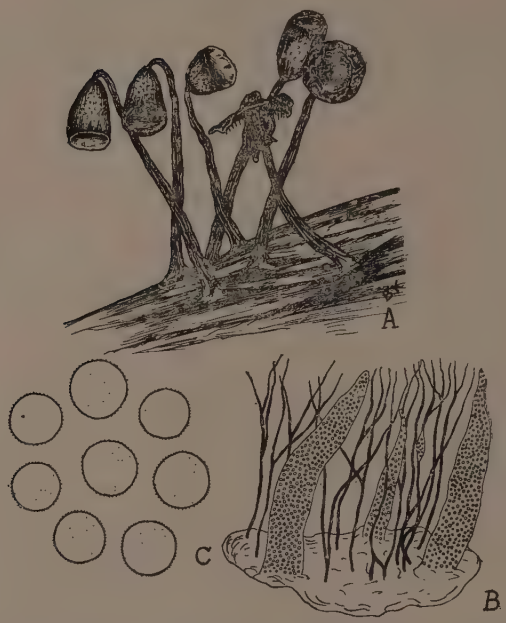
Spores 6 – 8 μ in diameter, black in a mass, violaceous brown under the microscope, globose to subglobose, very faintly verrucose.

Text-Fig. 6, A–C. (see page 104)

Collected on decaying wood, Saharanpur Road, Dehra Dun, August 4, 1954, **271**. On dead wood and a polypore, Saharanpur Road, Dehra Dun, August 5, 1953, **272**.

This species is characterized by the unique features of the peridium which is typically introverted at the top, from where it eventually ruptures in a lobate fashion. The petaloid lobes soon become reflexed exposing the yellow spiny processes (trabecules) and leaving the cylindrical inner wall protruding as a yellow pseudo-columella.

Collection n. 272 differs from collection n. 271 in the possession of longer and wider fructifications. Besides the colour of its sporangia is gray and that of trabeculae is white. Thus n. 271 is more typical of this species than n. 272.



Text-Fig. 6. *Physarella oblonga* (Berk. & Curt.) Morgan, A. Cup-shaped to subinfundibuliform sporangia due to introverted peridium with one sporangium showing petaloid lobes of the peridium and the cylindrical inner wall protruding as a pseudocolumella after dehiscence, X20. Note the numerous spiny processes or trabercules springing from the interior of the peridial lobes. B. Sparingly branching and anastomosing slender capillitial threads with a few small fusiform nodes and the stout spines or trabecules, both springing from the inner surface of the exterior walls of the peridium, X 320. C Very faintly verrucose spores, X1150.

74. *Didymium leoninum* Berk. & Br.

Fructifications sporangiate: sporangia 0.45 – 0.7 mm. in diameter, gregarious, or scattered, stipitate, erect, yellow, globose to subglobose, slightly umbilicate below: stipe 0.5 – 1 mm. long, brown, uniform in thickness throughout, roughened due to the deposition of lime crystals: hypothallus distinct, rotate, yellow, charged with yellow nodular lime crystals, venulose: peridium single, thick, tough or cartilaginous, deep reddish brown, covered over with yellowish, stellate lime crystals which are arranged usually in small discrete, scale-like clusters with naked blackish peridial areas in between marked by irregularly polygonal distinct ridges: dehiscence irregular, the peridium rupturing into various parts above while its lower part remaining persistent.

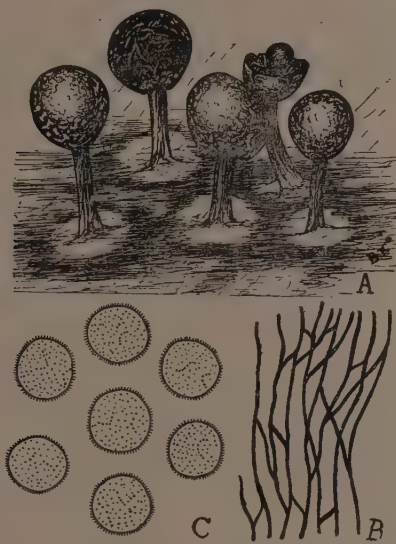
Columella distinct, knob-like or globose, orange, rough, persistent.

Capillitium abundant, composed of slender branching and anastomosing, purple brown threads becoming hyaline or colourless at the tips,

radiating from the columella and uniting with the peridium above.

Spores 7 – 9 μ in diameter, black in a mass, violet gray under the microscope, globose to subglobose, minutely and profusely verrucose, warts also showing a tendency to arrange in irregular rows.

Text-Fig. 7, A–C.



Text-Fig. 7. *Didymium leoninum* Berk. & Br. A. Fructifications, with one sporangium showing globose columella after dehiscence, X 20. B. Capillitium, X 320. C. Profusely and minutely verrucose spores showing a tendency to arrange in short irregular rows, X 1150.

Collected on dead leaves of *Mahonia* species, ferns, and other plants, Burning Ghat, Mussoorie, August 29, 1953, 273. New record in India.

This species is easily differentiated by the yellow coloured stipitate, slightly umbilicate sporangia, yellow globose columella, purple brown capillitial threads colourless at the apices, and violet gray, minutely verrucose, small spores (7 – 9 μ in diameter). The yellow lime crystals are charged all over on the peridium, stipe and hypothallus.

75. *Didymium clavus* (Alb. & Schw.) Rabenhorst.

Fructifications sporangiate: sporangia 0.5 – 0.7 mm. in diameter, gregarious or scattered, stipitate, erect, sometimes nodding, white, depressed-globose, strongly umbilicate below: stipe 0.5 – 1 mm. long and up to 0.25 mm. wide at the base, erect, or nodding, dark brown, darker below and lighter above, tapering upward, slightly longitudinally ridged

to almost smooth: hypothallus dark brown to almost black, rotate: peridium single, covered over densely with typically stellate white lime crystals except at the umbilicate base, dark gray, shining or somewhat iridescent: dehiscence irregular, the peridium rupturing from the top by longitudinal slits into petaloid lobes which remain joined together at the base.

Columella inconspicuous, represented merely by the raised basal part of the sporangium, light brown and shining.

Capillitium abundant, composed of purple-violet, thin, sparsely branched and sparsely anastomosed, noncalcareous threads with hyaline, attenuated extremeties.

Spores 5.6 – 6.4 μ in diameter, black in a mass, violaceous brown under the microscope, globose to subglobose, inconspicuously verrucose.

Text-Fig. 8, A–C.

Text-Fig. 8. *Didymium clavus* (Alb. & Schow.) Rab. A. Depressed globose sporangia umbilicate below, X20. B. Capillitium, X 320. C. Minutely verrucose, small spores, X 1150.

Collected on dead wood of a tree and on a living moss, Chakrata Toll, Mussoorie, July 23, 1954, **274**.

The spores of this Mussoorie collection are rather small for the species. The small size is due to the fact that they were mostly empty and hence shrunk. This often happens when fructifications are wet after maturity as it was probably the case with the Mussoorie collection. The species recorded from Southern India by Agnihothrudu, 1956, is reported to possess normal sized spores.

76. *Diderma hemisphaericum* (Bull.) Hornem.

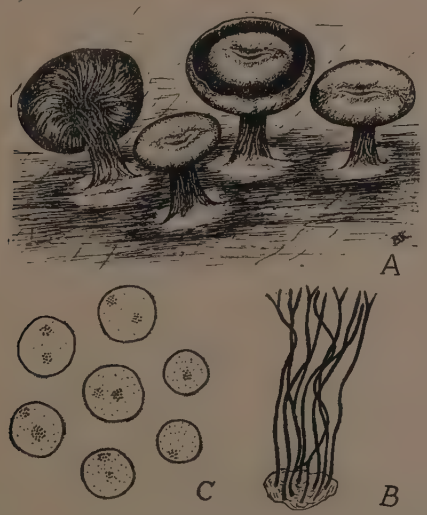
Fructifications sporangiate: sporangia 0.5 – 1.25 mm. in diameter, gregarious, sometimes confluent, stipitate, erect, chalk-white, sometimes light-violet or pinkish when lower surface somewhat darker than the upper surface, orbicular, typically lenticular as sporangia are strongly compressed or flattened, depressed above, umbilicate below: stipe 0.38 – 1.5 mm. long and up to 0.16 mm. wide, light cream coloured or whitish below and brown upward, erect, or rarely bent, stout, thick, uniform width or sometimes tapering upward, expanded above into the pileate sporangium, longitudinally rugulose or wrinkled, the wrinkles continuing like radial veins on the lower surface of the sporangium, calcareous: hypothallus-small, white, sometimes light dull yellow, calcareous, rotate, sometimes confluent: peridium double: outer peridium white or sometimes violaceous, crustose or shell-like, calcareous, fragile, closely applied to the inner peridium: inner peridium thin, delicate, dark gray or cinereous, iridescent: dehiscence irregular to circumscissile, the outer peridium at first rupturing along the margin, followed by irregular rupturing of the inner peridium. The stipes supporting the columella and the lower portion of the peridium remain persistent long after dehiscence.

Columella not distinct, represented only by the slightly raised base of the sporangium, light brown, usually bright or shining, calcareous.

Capillitium abundant to scanty, composed of delicate, slender, hyaline, noncalcareous, sparsely branching (usually at the distal ends) and rarely anastomosing very fine threads.

Spores 7 – 10 μ in diameter, black in a mass, violaceous brown under the microscope, globose to subglobose, minutely verrucose, warts sometimes also aggregated into clusters.

Text-Fig. 9, A–C.



Text-Fig. 9. *Diderma hemisphaericum* (Bull.) Nornem. A. Lenticular stipitate sporangia, X 20. B. Capillitium , X320. C. Profusely and minutely verrucose spores showing a few clusters of darker and thicker warts, X 1150.

Collected on dead leaves and dead twigs, Mossy Fall, Mussoorie, July 19, 1954, **275**. On dead leaves and twigs, Forest Research Institute, Dehra Dun, August 20, 1953, **276**.

This excellent species is easily differentiated by its remarkable lenticular stipitate sporangia. The colour of the fructifications is typical chalk-white as is the case with collection n. 275. The spore size of n. 275

was rather small (6.4–8 μ in diameter) for the species. This may be due to the fact that the spores were mostly empty and more or less collapsed at the time of its collection. Collection n. 276 is remarkable in having light violet or pinkish sporangia, which colour is not reported for the species. Hence it may be regarded as a pink form of *D. hemisphaericum*. The spores of this collection (n. 276) are also much bigger (7.5 – 12 μ in diameter) than those of n.275. However, this large size is due to the presence of few oval spores up to 11 or 11.5 (–12) μ, but they are correspondingly narrower. There are no normally matured globose spores over 10 μ in diameter.

Agnihothrudu, 1954, who collected the species from half a dozen localities in Southern India describes the spores as smooth. It is very doubtful if they are entirely smooth. In most probability his collections possess nearly smooth (or inconspicuously verrucose) spores.

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REFRIGERATION OF CULTURES OF *SCLEROTINIA SCLEROTIORUM* (Lib.) de Bary AS A STIMULUS TO THE PRODUCTION OF A SECOND CROP OF SCLEROTIA

KISHAN SINGH BEDI

(Accepted for publication September 10, 1958)

It has been pointed out already (Bedi, 1958) that the cultures of the sclerotial Punjab race of *Sclerotinia sclerotiorum* (Lib.) de Bary produce only one ring of sclerotia at the periphery against the glass edge of the receptacle on media of moderate richness, like the potato-dextrose agar. Presumably, the main central part of the cultures without sclerotia still has a considerable amount of unused nutrients. To be able to induce the cultures to utilize this un-used reserve of food materials for producing a new crop of sclerotia was considered a matter of great scientific significance. Among the several methods tried by the author to accomplish this object, the refrigeration of the cultures at sub-zero temperatures, after they had produced one usual ring of sclerotia at the periphery, did the job in a remarkable manner. Also, it was considered worth-while to ascertain, if the cultures of the non-sclerotial Canadian mutant of this fungus could be stimulated to form sclerotia, when refrigerated. For these studies, both the races were grown at 25 °C. on potato-dextrose agar. The sclerotial Punjab race produced its full crop of sclerotia in the form of a peripheral ring in about a week as usual, while the mycelial Canadian mutant race produced none. The cultures of both the races, when 9 days old, were subjected to —2 °C., and —25 °C. in two separate deep-freeze cabinets for periods of 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 days. For each period of exposure to each of the two sub-zero temperatures, there were triplicate cultures of each race. Cultures to serve as controls were kept constantly at 25 °C., which is the optimum temperature for their growth. After each period of exposure to the two sub-zero temperatures, the cultures were restored to 25 °C.

The data pertaining to this experiment are presented in table 1 and the photographs of the cultures of the sclerotial Punjab race subjected for different periods to —2 °C., and —25 °C., alongwith the cultures, kept constantly at 25 °C. to serve as control, are shown in fig. 1.

The data and the photographs reveal very striking results. A new crop of sclerotia has developed in all the cultures of the Punjab race refrigerated at —25 °C., whether the period of exposure to this temperature is only one day, or is as long as 28 days. In the case of its cultures refrigerated at —2 °C., a new crop of sclerotia has been produced only as the result of prolonged exposure of 21 and 28 days. This clearly shows that what a very small period of exposure to the very low temperature of —25 °C. can accomplish, is accomplished by a much longer period of exposure at the relatively high refrigeration temperature of —2 °C. No new crop of sclerotia was produced in the un-refrigerated control cultures kept

TABLE 1. Effect of refrigeration of 9-day-old cultures of the sclerotial Punjab race and the mycelial Canadian mutant race of *Sclerotinia sclerotiorum* for periods of time ranging from 1 to 28 days on sclerotial formation

	Exposure of cultures to −2 °C in days										Exposure of cultures to −25 °C in days									
	1	2	3	4	5	6	7	14	21	28	1	2	3	4	5	6	7	14	21	28
Punjab race Number* of sclerotia in the new crop, produced as the result of refrigeration.	0	0	0	0	0	0	0	0	10.3	13	7.3	9	13.3	13	16	13.6	13.6	16.3	14	13.3
Number* of sclerotia in cultures before refrigeration.	31	34.6	32	31.6	32.6	31.3	33.3	33.6	32	30.6	31.3	31	31	32	32.6	32	32.6	32	31.6	31.3
The new crop of sclerotia expressed as percentage of the old.	...	...	...	...	...	...	...	...	32.2	42.4	23.3	29	42.9	40.6	49	42.5	41.7	50.9	44.3	42.1
Canadian Mutant	The race, which is non-sclerotial, could not be stimulated to produce sclerotia by the refrigeration of its cultures at −2 °C and −25 °C.																			

Continued overleaf

*Average of 3 replicates.

Control cultures were kept constantly at 25 °C. in the case of the Punjab race. They produced only one crop of sclerotia in the form of a peripheral ring as usual, no new crop of sclerotia being formed throughout the entire course of the experiment. Control cultures of the Canadian mutant race produced sclerotia neither before, nor after refrigeration.

Average size, as based on 100 random measurements of sclerotia in the original crop : .....5.2 x 2.5 mm.

Average size, as based on 100 random measurements of sclerotia in the new crop, produced as the result of refrigeration : .....6.0 x 4.5 mm.

Weight of 100 random sclerotia taken from the original crop : .....610 mg.

Weight of 100 random sclerotia taken from the new crop produced as the result of refrigeration : .....1251 mg.

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constantly at 25 °C. It may be noticed that the number of sclerotia in the new crop, wherever formed in the series, constitutes a considerable percentage of the original crop. Also it should be observed that the size and the weight of sclerotia, produced as the result of cold treatment, has been almost doubled. The increase in the number of sclerotia consequent upon the exposure of cultures to sub-zero temperatures is, in most cases, more than 40 per cent, and when the weight of the sclerotia ,which has been almost doubled, is taken into consideration, the stimulatory effect of refrigeration of the cultures is easily reflected in about 80 per cent increase in sclerotial production on dry-weight basis. Such an increase is very remarkable. It was noticed that the cultures, in which a new crop of sclerotia had been produced as the result of refrigeration, there was first an abundant development of white-fluffy mycelium in the central part of the colonies, which ordinarily has only a very thin mat of creeping mycelium unable to support the development of any sclerotia. The thick fluffy mycelial growth soon became transformed into the raised-up, white, beadlike structures, eventually turning black to form the hard resting bodies, the sclerotia. An examination of the photographs of the cultures, which have produced the new crop of sclerotia, shows that, even after the formation of this new crop, there still remains behind a considerable amount of the fluffy mycelium, which had developed as the result of the cold treatment. In cold countries, where the plant debris infected with *Sclerotinia sclerotiorum* gets buried under snow during winter, the pathogen, instead of suffering damage due to protracted freezing, is expected to be stimulated as a result thereof and to produce a new crop of sclerotia, when the snow melts during the spring and the season warms up. Such an observation has been actually recorded by Young (1934). He noticed that cankers caused by *S. sclerotiorum* on holly-hock stems were originally without sclerotia, but after they had been under snow during winter, sclerotia developed during spring on these cankers. For this phenomenon, he did not offer any explanation, which the writer's results now furnish.

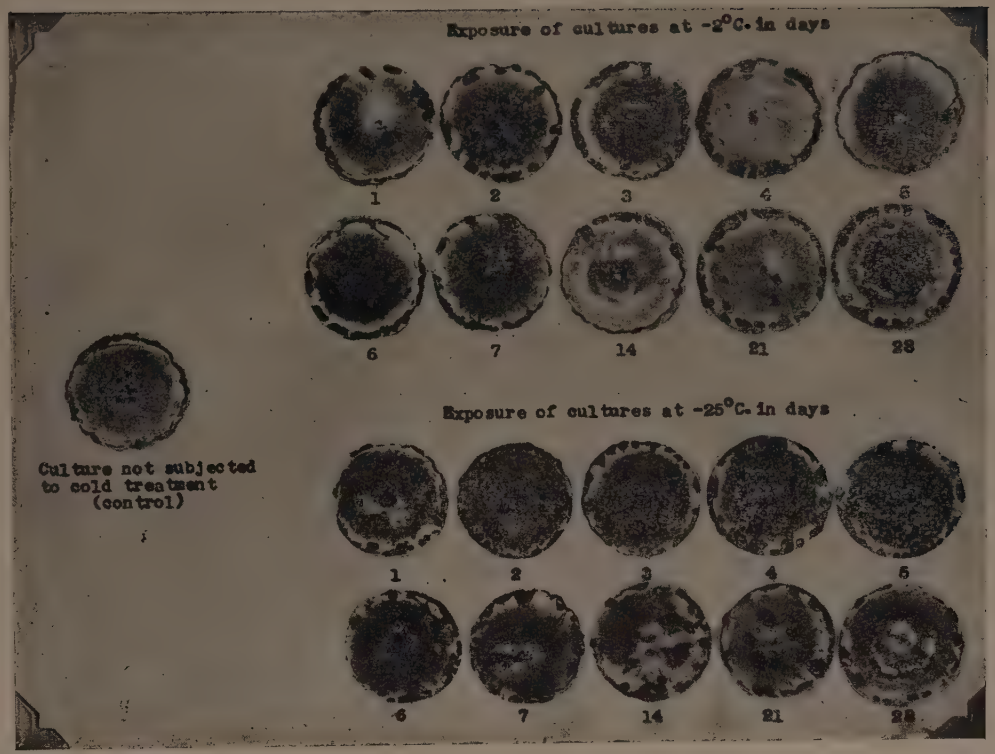

Fig. 1. Effect of freezing the 9-day-old cultures of the sclerotial Punjab race of *Sclerotinia sclerotiorum* at –2 °C., and –25 °C. for 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 days after the formation of a normal crop of sclerotia in the form of a ring at the periphery. Notice the development of a new crop of sclerotia in all the cultures as the result of refrigeration at –25 °C., whether for one day, or for 28 days and subsequent restoration to 25 °C., the optimum temperature for the growth of the fungus. Under the influence of the relatively high temperature of –2 °C., exposures of 21 and 28 days only are capable of inducing a new crop of sclerotia in the cultures.

Fig. 2. The Canadian mycelial mutant race, showing a more fluffy and whiter sclerotial mutant with three well-defined black sclerotia. The mutant appeared in a culture, which was frozen at –25 °C. for 3 weeks, and subsequently restored at 25 °C.

Whereas the refrigeration treatment has stimulated the cultures of the sclerotial Punjab race to produce a new crop of sclerotia, it has failed to induce the mycelial Canadian mutant to form even one crop of sclerotia. However, in one of the culture plates of this race, subjected to –25 °C. for 3 weeks and subsequently restored to 25 °C., which temperature is optimum for its growth, there suddenly appeared a mutant (Fig. 2) overgrowing the parent culture from one side and producing some typical black sclerotia. Though the new mutant is not exactly identical with the original sclerotial parent culture from sunflower, obtained from Canada and which had given rise to the mycelial mutant, yet the writer thinks that on account of the fundamental similarity of this new sclerotial mutant (arising from the mycelial mutant race) with the original parent culture from sunflower, this is possibly a case of reversible mutation induced by a prolonged exposure to the very low temperature of —25 °C.

Summary

The cultures of the sclerotial Punjab race of *Sclerotinia sclerotiorum* (Lib.) de Bary growing in glass receptacles, such as Petri plates and conical flasks, ordinarily produce only one ring of sclerotia at the periphery on media of moderate richness, like the potato-dextrose agar. Most of the colony surface, which has a very thin mat of creeping mycelium, remains devoid of any sclerotia despite the presence of a large un-used reserve of nutrients in the medium. The refrigeration of cultures of this race at the sub-zero temperature of –25 °C. for any period ranging from 1 to 28 days stimulates the central part of the cultures to produce an abundant amount of mycelium, which gives rise to a new crop of sclerotia. These sclerotia, in most cases, constitute about 80 per cent of the original crop at the periphery. In the case of refrigeration at the relatively high temperature of –2 °C., a new crop of sclerotia is produced only in the wake of prolonged exposure of 3 and 4 weeks. Thus, what is accomplished by a

very brief period of one day at −25 °C., is accomplished by 3 to 4 weeks at the relatively high temperature of −2 °C.

The sclerotia, produced as the result of refrigeration, are almost twice as large and twice as heavy as those produced in the absence of this treatment.

Acknowledgement. The work was carried out in the Division of Plant Pathology, College of Agriculture, University Farm, St. Paul, Minnesota, U.S.A., and forms a very small part of the thesis submitted for the degree of Doctor of Philosophy. The wirter is extremely indebted to Dr. Elvin Charles Stakman, the then Head of the Division of Plant Pathology and Botany, and now Professor Emeritus, under whose inspiring guidance the work was pursued.

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STUDIES ON THE FUNGICIDAL CONTROL OF RICE BLAST

1. THE FUNGISTATIC EFFICACY OF CERTAIN ORGANIC AND INORGANIC FUNGICIDES ON *PIRICULARIA ORYZAE*.

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(Accepted for publication October 5, 1958)

Introduction. Rice blast, caused by the fungus *Piricularia oryzae*, is the most serious disease affecting the rice plant in Ceylon. This disease is characterised by the appearance of spots on the leaves and glumes, by the breaking over of the 'necks' and branches of the panicles and by the blighting or blasting of the entire panicles. All these phases of blast can be highly destructive and may sometimes lead to considerable loss of the crop.

Foliage and panicle infection may be kept at bay by the application of protective fungicides to the plant surface. Although chemical control of blast has been recognised for a long time, little work has been done in the search for new and more effective fungicides in the control of the disease. The main objective of the present laboratory investigation was, therefore, to determine the fungistatic efficacy of a wide range of organic and inorganic fungicidal spray formulations on *P. oryzae*. Nisikado *et al* (1951) sprayed glass slides with different fungicides and assesed the inhibition of spore germination of *P. oryzae*. They found that only Bordeaux mixture at 0.4 per cent concentration inhibited spore germination.

Materials and Methods. The method of assay used in the present investigation is in many respects similar to that described by Montgomery and Moore(1937). The glass slides used were treated with the following cleaning solutions in order to ensure the complete removal of any foreign matter; warm dilute nitric acid, hot 2.5 per cent caustic soda, chromic acid cleaning solution, dilute 'Teepol' finally rinsed in several changes of demineralised water. A 'Crystalab Deeminizer' was used for demineralisation of water.

An impression of a circle with an internal diameter of 12 mm. was obtained on a glass slide with a solution of cellulose acetate in acetone; three such circles were made on each 3" x 1" slide. 0.015 ml. of the test material was pipetted into each circular area. It was evenly spread by means of a glass needle and allowed to dry at the room temperature for 24 hours.

A vigorously growing isolate of *P. oryzae* was cultured in the following medium, the composition of which is given below:

Quaker oats	40	gm.
Brewers Yeast	15	gm.
Berin (aneurin hydrochloride)	0.2	gm.
Agar	20	gm.
Water	1,000	c.c.

On this medium abundant spores were produced. Spores from 6 – 10 day old cultures gave consistent and good germination. In preliminary germination tests with *P. oryzae*, germ tube initiation was noted after an hour of incubation at the room temperature and in a period of three hours 100 per cent of the spores germinated. In this study, however, germination of the spores was estimated after an incubation period of 24 hours.

0.04 ml. of a standard spore suspension was evenly sown in each circular area within which a deposit of fungicide had been obtained and the germinability of the spores in contact with the fungicide estimated. Absence of germ tube initiation after the incubation period at room temperature was taken as a reliable criterion of inhibition of spore germination.

All spore suspensions and solutions or suspensions of fungicides were prepared with demineralized water, the impurities of which were not more than 0.25 p.p.m. when measured as NaCl.

The following fungicides were used in the study:-

Fungicide.	*Active ingredient.*
Cupravit	Copper oxychloride
Shell Copper	Copper oxychloride
Blitox	Copper oxychloride
Perenox	Cuprous oxide
Copper Sandoz	Cuprous oxide
Bordeaux Mixture 1.	Copper sulphate 5 : Lime 5 : Water 100.
Bordeaux Mixture 2.	Copper sulphate 8 : Lime 5 : Water 100.
Bordeaux Mixture 3.	Copper sulphate 5 : Lime 8 : Water 100.
Thiovit	25–28% Polysulphides (wettable)
2 per cent Ceresan	Ethyl mercury chloride
Tillex	Ethyl mercury chloride
Dithane Z–78	65% Zinc ethylene bisdithiocarbamate.
Zerlate	76% Zinc dimethyl dithiocarbamate.
Fermate	76% Ferric dimethyl dithiocarbamate.
Dithane M 22	70% Manganese ethylene bisdithiocarbamate.
Karathane	22.5% Dinitro (1–methyl heptyl) phenyl crotonate plus dinitrophenol and derivative.

Experimental Results and Discussion. Tables 1 and 2 show the percentages of inhibition of spore germination of *P. oryzae* with various concentrations of the fungicides listed above. The results were analysed in the manner described by Bliss (1935). Inhibition of spore germination, expressed as probits, were plotted against log. concentrations of the fungicide, and in every case these were found to be linearly related. The dosage response curves for the inorganic and organic fungicides are given separately in Figs. 1 and 2 respectively. The regression equations were computed and are given together with other data in Table 3. The relative effectiveness of the fungicides was compared by calculating the ED 50. The results shown in Table 1 indicate that among the copper fungicides tested, the copper oxychloride formulations were weakly fungistatic and that further exploration was unnecessary with this group of compounds.

TABLE 1. Percentage of spores of *Piricularia oryzae* inhibited by various inorganic fungicides

Fungicide	PERCENTAGE CONCENTRATION									
	0.5	0.3	0.1	0.08	0.06	0.05	0.04	0.03	0.025	0.02	0.01
Shell Copper	0	0.4	0	—	—	0.7	—	—	0	—	—
Cupravit	1.0	0	0.4	—	—	0	—	—	0	—	—
Blitox	3.4	0.7	0	—	—	0	—	—	0	—	—
Perenox	100	84.0	66.7	—	—	44.7	—	—	27.0	—	—
Copper Sandoz	—	—	89.0	75.7	69.4	—	50.4	—	—	7.0	4.0
Bordeaux Mixture 1	—	97.4	87.4	—	—	77.4	—	39.4	—	—	—
Bordeaux Mixture 2	95.4	92.0	83.4	—	—	57.4	—	—	25.7	—	—
Bordeaux Mixture 3	89.0	83.7	64.4	—	—	46.0	—	—	19.4	—	—
Thiovit	93.0	76.4	16.7	—	—	4.0	—	—	—	—	—

TABLE 2. Percentage of spores of *Piricularia oryzae* inhibited by various organic fungicides

Fungicide	PERCENTAGE CONCENTRATION													
	0.4	0.035	0.03	0.025	0.02	0.015	0.01	0.008	0.006	0.004	0.002	0.0016	0.0013	0.001	0.0008
Ceresan	98.7	96.0	86.0	76.0	40.4	8.0	—	—	—	—	—	—	—	—	—
Tillex	94.7	—	86.4	—	79.7	—	65.4	53.7	33.0	—	—	—	—	—	—
Dithane Z-78	—	—	—	—	97.0	—	82.4	38.7	17.4	—	—	—	—	—	—
Zerlate	—	—	—	—	—	—	100	98.7	93.0	72.4	25.7	—	—	—	—
Fermate	—	—	—	—	—	—	—	—	—	—	80.4	45.4	17.7	8.7	2.7
Dithane M-22	—	—	—	—	—	—	87.7	48.0	82.4	1.4	—	—	—	—	—
Karathane	—	—	—	—	—	—	79.7	93.4	11.4	—	10.7	—	—	—	—

TABLE 3. Analysis of Results

Fungicides	Equations of Probit Lines	n	b	Log E D 50	E D 50 Per cent
Thiovit	Y=3.35x — 2.580	2	3.35 ± 0.142	2.265 ± 0.063	0.1842
Perenox	Y=1.76x + 1.886	2	1.76 ± 0.239	1.766 ± 0.355	0.0583
Copper Sandoz	Y=3.29x — 0.418	4	3.29 ± 0.334	1.645 ± 0.073	0.0441
Bordeaux Mixture 1	Y=2.45x + 1.317	2	2.45 ± 0.877	1.505 ± 0.338	0.0320
Bordeaux Mixture 2	Y=1.83x + 1.993	3	1.83 ± 0.281	1.640 ± 0.293	0.0437
Bordeaux Mixture 3	Y=1.54x + 2.184	3	1.54 ± 0.195	1.833 ± 0.166	0.0681
Ceresan	Y=8.27x —14.260	4	8.27 ± 0.150	2.333 ± 0.033	0.0215
Tillex	Y=2.17x + 0 877	4	2.17 ± 0.243	1.899 ± 0.113	0.0079
Dithane Z–78	Y=6.48x — 7.40	2	6.48 ± 1.590	1.915 ± 0.252	0.0082
Zerlate	Y=4.69x — 1.829	3	4.69 ± 0.255	1.456 ± 0.046	0.0029
Fermate	Y=7.35x — 3.885	3	7.35 ± 0.809	1.204 ± 0.013	0.0016
Dithane M–22	Y=9.61x —13.216	2	9.61 ± 1.197	1.899 ± 0.644	0.0079
Karathane	Y=4.60x — 2.232	3	4.60 ± 0.029	1.573 ± 0.009	0.0037

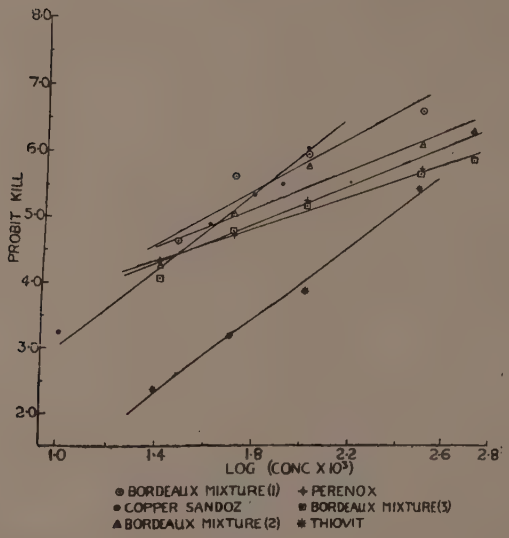

Fig. 1. Probit regression lines for certain inorganic fungicides against *P. oryzae*.

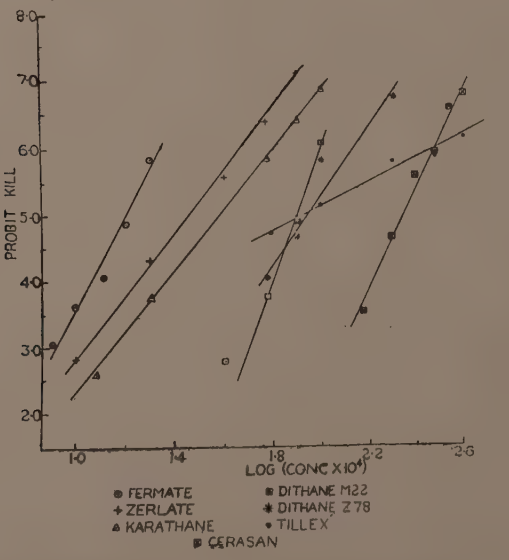

Fig. 2. Probit regression lines for certain organic fungicides against *P. oryzae*.

A marked distinction in the fungistatic activity of copper oxide and copper oxychloride formulations was observed; for example, all copper oxychlorides tested had little or no fungistatic activity on spore germination of *P. oryzae* even at a relatively high concentration of 0.5 per cent, whereas the cuprous oxide gave maximum inhibition at this concentration.

All three preparations of Bordeaux mixture inhibited spore germination of *P. oryzae*. The increase in the copper content and the quantity of lime in Bordeaux mixture does not appear to contribute to the fungistatic activity of the preparation. At the level of 50 per cent inhibition Bordeaux mixture (1) was the best of all the copper formulations tested. Perenox is slightly less effective than Copper Sandoz.

The most toxic formulation among the dithiocarbamates was Fermate and by far the best fungicide.

Summary

With the aid of a laboratory spore germination technique, a number of inorganic and organic fungicides have been tested against the fungus *Piricularia oryzae*.

Dosage-mortality curves have been plotted for these fungicides and the ED 50 values computed.

All copper oxychloride formulations were ineffective whereas cuprous oxides and Bordeaux mixture preparations proved fungistatic.

The organic fungicides were superior to the inorganic ones. Among those tested Fermate had a low ED 50 value and was by far the most effective fungicide.

Acknowledgments. The authors are indebted to Mr. E. M. B. de Silva for the technical assistance. They also wish to thank Mr. D. M. Rodrigo for his advice on the statistical work.

Department of Agriculture,
Peradeniya, Ceylon.

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THREE NEW RECORDS OF FUNGI IMPERFECTI ZINGIBERACEAE

M. A. Salam, P. N. Rao and P. Rama Rao

(Accepted for publication October 10, 1958)

The genus *Piricularia* was established by Saccardo (1880) which includes a number of parasitic species infecting several graminaceous hosts of which *P. oryzae* is very important inciting 'blast' disease of paddy. Uptil now *Piricularia* species have been reported on members of Gramineae, Cyperaceae, Zingiberaceae (Luttrell, 1954) and recently on Commelinaceae (Thirumalachar *et al* 1956). In the present paper a brief account of morphology of three Fungi Imperfecti collected on *Curcuma montana* Rosex. and *Costus speciosa* Sm. from Pakhal forest in Hyderabad State, (Andhra Pradesh), is presented.

1. *Piricularia* sp. on *Curcuma montana.*

Infection spots epiphyllous, circular or in concentric rings, pale brown, with a white centre, circular then elliptical, measuring 10–15 mm. in diameter; more than 100 spots on each leaf in case of severe infections; fruiting abundantly. Mycelium intercellular, hyaline, septate, 3–5 μ in diameter. Conidiophores sub-hyaline, unseptate, straight, geniculate bearing a number of conidia acro-pleurogenously. Conidia hyaline, pyriform, mostly 2–septate, not constricted at septa, middle cell slightly larger and end cells narrower, with a small hilum at the base showing the point of attachment, measuring 14.4–27.2 x 6.4–8.0 μ (19.1 x 7.4μ).

Habit. On leaves of *Curcuma montana* Rosex. (Zingiberaceae), Pakhal forest, Warangal District, 28–9–56, P. Rama Rao, O. U. B., Herb. 'Hy' No. 58.

The present fungus in all its morphological characters i.e. in size and shape (pyriform) of the conidia resembles *P. grisea* (Cke.) Sacc.

2. *Piricularia* sp. on *Costus speciosa*

Infection spots small circular, gradually becoming water soaked, measuring 8–10 mm. in diameter. Mycelium intercellular, hyaline, septate, 4–6 μ in diameter. Conidiophores sub-hyaline, unseptate, straight, bearing 3–4 conidia at the tip. Mature conidia hyaline, obclavate, 1–2 septate, mostly 2–septate with a short hilum at the base, measuring 14.4–24.8 x 6.4 – 12.0 μ (19.6 x 8.5 μ).

Habit. On the living leaves of *Costus speciosa* Sm. (Zingiberaceae), Pakhal Forest, Warangal district, 28.9.56, M.A. Salam, O.U.B. Herb. 'Hy' No. 59.

Symptoms caused by the fungus are quite different from those caused on *Curcuma montana.*

On the basis of the conidial shape (obclavate) and measurements, the present fungus agrees closely with *P. oryzae* Cav.

3. *Helicomina costi* sp. nov. Salam & Rao, P.N.

The fungus is associated with the infection spots of *Piricularia* sp. producing sooty patches. Colonies are hypophyllous extending over the entire spots giving a dark, silky appearance, measuring 8–12 mm. in diameter. Conidiophores are dark brown, elongate, straight, unbranched, simple, occurring in compact divergent clusters (4–20) arising from a pale hypophyllous stroma; septate, constricted at septa, with a pale brown swollen base and rounded hyaline apex measuring 270.0 – 414.0 x 4.8 μ. Conidia light brown, cylindrical, straight or curved, 1–9 septate, not constricted at septa, with a small hilum at the base, developing singly and acrogenously at the tips of the conidiophores with a broad or narrowly rounded smooth tip measuring 27.2 – 72.0 x 6.4 – 7.2 μ germinating at both ends.

Habit. On the living leaves of *Costus speciosa* Sm. (Zingiberaceae), Pakhal forest, Warangal District, 28.9.56, M.A. Salam, O.U.B. Herb. 'Hy' No. 60.

The fungus belongs to Phragmosporous Dematiaceae, but it differs from *H. caperoniae* and *H. indica* reported by Olive (1948), Subramanian (1956) respectively in the large size of the conidia and long pluriseptate conidiophores, as such it was presented as a new species.

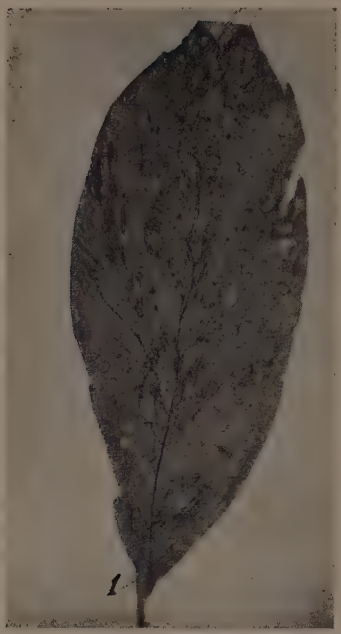
Hemiconina costi spec. nov. Salam & Rao, P.N.

Fungus, atque occurrit in maculis iam efformatis a specie quadam *Piriculariae*, productique maculas carbonaceas. Coloniae extenduntur per totam superficiem macularum priorum, quas fusce sericas reddunt. Coloniae sunt hypophyllae, diametientes 8 – 12 mm. Conidiophori fusce brunnel, elongati, recti, haud ramosi, simplices, occurrentes in massis compactis et divergentibus, surgentes e stromate pallide brunneo et hypophyllo, 4–20–septati, constricti ad septa, basi pallide brunnea et tumida, apice rotundato hyalino, magnit. 270.0 – 414 x 4.8 μ. Conidia pallide brunnea, cylindrica, recta vel curva, 1–9–septata, non constricta, hilo parvo ad basin ornata, evoluta singulariter et acrogene ad apices condiophororum, apice anguste vel late rotundato et levi, magnit. 27.2 – 72.0 x 6.4 – 7.2 μ, germinantia ad utrumque apicem.

Typus lectus in foliis viventibus *Costi speciosi* Sm. a familia Zingiberacearum, in sliva ad Pakhal, in regione Warangal, die 28 Septembris anni 1956, a M.A. Salam. et Positus in O.U.B. Herb 'Hy' Sub numero, 60.

Duplicate set of the herbarium of the new sp. has been deposited in the Herb. Crypt. Ind. Orient of the Agricultural Research Institute, New Delhi.

Acknowledgments. The authors wish to thank Prof. M.



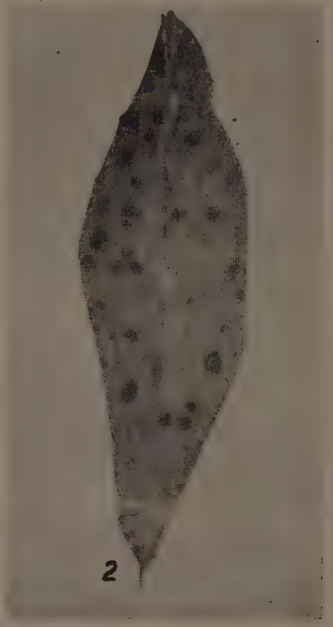


Fig. 1. Leaf of *Curcuma montana* showing infection spots.

Fig. 2. Leaf of *Costus specisa* showing infection spots, covered by a hyperparasite.

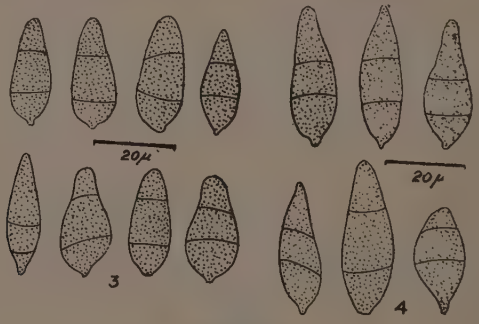
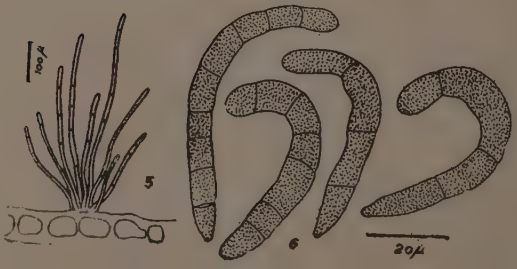


Fig. 3.Conidia of *Piricularia* sp. on *Curcuma montana*.

Fig. 4. Conidia of *Piricularia* sp. on *C. speciosa*.

Fig. 5. Conidiophores and conidia of *Helicomina costi*.



Sayeeduddin for the encouragement and to Rev. Father Prof. H. Santapau for the Latin translation of the new species.

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Osmania University, Hyderabad-Dn.

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FUNGI FROM HYDERABAD (INDIA) – II
(OIDIUM SPP)

M. A. Salam and P. N. Rao

(Accepted for publication October 10, 1958)

The material for this paper was collected during the winter months of 1955 from the Hyderabad State. Out of sixteen species described, eleven marked with astericks are new records to India.

*1. *Oidium* sp.

Mycelium superficial, creeping, branched, subhyaline, septate at regular intervals, measuring 4.0 – 6.2μ in diameter, forming white coating on the upper surface of the leaves. Conidiophores erect, simple, unseptate, bearing 2 – 3 conidia in a chain which drop off soon. Conidia hyaline, single celled, ellipsoidal, simple, with rounded ends, measuring 24.0 – 38.4x 11.2 – 16.0μ.

Habit. On the living leaves of *Tephrosia purpurea* Pers. (Papilionaceae), University Campus, 10–10–55, P. N. Rao. O. U. B. Herb. 'Hy' No. 62.

*2. *Oidium* sp.

Mycelium superficial, creeping, hyaline, septate, branched, measuring 4.8 – 8.0μ in diameter, forming thick-white coating on both sides of the leaves. Conidiophores hyaline, septate, mostly straight. Conidia hyaline, 1–celled, with slightly flattened ends, elliptical, bulging in the middle, measuring 27.2 – 34.0 x 11.2 – 13.2μ.

Habit. On the living leaves of *Tamarindus indica* L. (Caesalpinaceae), Hyderabad, 1–1–56, M.A. Salam. O.U.B. Herb. 'Hy' No. 63.

*3. *Oidium* sp.

Mycelium slender, superficial, hyaline, septate, creeping, branched, forming white cottony patches on both sides of the leaves, measuring 4.8–7.2μ in diameter. Conidiophores hyaline, straight, unseptate, bearing 3–4 conidia at the tip. Conidia hyaline, long, ellipsoidal, 1-celled with rounded ends, some broader in the middle measuring 22.4 – 44.6 x 11.2 – 16.0μ.

Habit. On the living leaves of *Santalum album* L. (Santalaceae), Hyderabad, 2–1–56, O.U.B. Herb. 'Hy' No. 64.

*4. *Oidium* sp.

Mycelium superficial, creeping, hyaline, septate, branched, forming white coating only on the upper surface of the leaves, measuring 4.8 – 8.0μ

in diameter. Conidiophores hyaline, straight, unseptate, bearing 4–6 conidia in a chain. Conidia hyaline, 1–celled, slightly elliptical, with flat ends, measuring 24.0 – 33.6 x 12.8 – 17.6μ.

HABIT. On the living leaves of *Melothria maderaspatana* Cogn, (Cucurbitaceae), Narsapur Forest, 6–11–55, P. N. Rao, O. U. B. Herb. 'Hy'' No. 65.

*5. *Oidium* sp.

Mycelium superficial forming cottony white patches on both surfaces of the leaves, hyaline, septate, indistinctly branched, measuring 4.0 – 6.5μ in diameter. Conidiophores hyaline, unseptate, straight, bearing 4–5 conidia in a chain. Conidia hyaline, 1–celled, simple elliptical with blunt ends, measuring 24.0–48.0 x 14.4 – 17.6μ.

HABIT. On the living leaves of *Ipomoea obscura* K. Gawl. (Convolvulaceae), Narsapur forest, 6–11–55, P. N. Rao, O. U. B. Herb. 'Hy' No. 66.

*6. *Oidium* sp.

Mycelium superficial, forming white patches, hyaline, septate, branched, measuring 3.2 – 5.6μ in diameter. Conidiophores hyaline, straight, unseptate, bearing 4 – 6 conidia in a long chain. Conidia hyaline, 1–celled, globose to elliptical, giving bulged appearance with blunt ends, measuring 19.2 – 35.2 x 12.8 – 17.6 μ. Perfect stage has been collected after sending the paper to the press. It will be reported subsequently.

HABIT. On the living leaves of *Pedilanthus tithymaloides* Poit. (Euphorbiaceae), Adigmet, 17–12–55, P. N. Rao. O. U. B. Herb 'Hy' No. 67.

*7. *Oidium* sp.

Mycelium superficial, creeping, hyaline, septate, branched, forming cottony white patches only on the upper surface of the leaves, measuring 3.8 – 6.0μ in diameter. Conidiophores hyaline, straight, 2 – 3 septate, bearing 2 – 6 conidia in a chain. Conidia hyaline, 1–celled, small, smooth, elliptical with rounded ends measuring 14.4 – 19.2 x 6.4 –8.0μ.

HABIT. On the living leaves of *Phyllanthus rheedii* W. (Euphorbiaceae), Agriculture Farm, 15–1–56, P. N. Rao. O. U. B. Herb. 'Hy' No. 68.

*8. *Oidium* sp.

Mycelium superficial, creeping, hyaline, septate, branched, measuring 3.2 – 6.0μ in diameter. Conidiophores hyaline, 3–4 septate, cylindrical, bearing 3–4 conidia in a chain. Conidia hyaline, 1–celled, more or less rectangular, with flat ends, measuring 30.4 – 40.0 x 12.8 – 16.0μ.

HABIT. On the living leaves of *Pergularia extensa* N. E. Br. (Asclepiadaceae), University Campus, 1–1–56, P.N. Rao, O.U.B. Herb. 'Hy' No. 69.

9. *Oidium cyparissiae* Syd.

Sydow, H. *Hedwigia*, P. 163, 1897.

Saccardo, P. A. *Syll. Fung.* **14** : 1041, 1899.

Patel, M. K., Kamat, M. N. and Bhide, V. P. *Indian Phytopath.* **2** : 142–143, 1944.

HABIT. On the living leaves of *Euphorbia hirta* L. (Euphorbiaceae), Adigmet, 12–9–55, P. N. Rao, O.U.B. Herb. 'Hy' No. 70.

10. *Oidium acalyphae* Chiddarwar.

Chiddarwar, P.P., *Lloydia*, **18** : 46–47, 1955.

HABIT On the living leaves of *Acalypha ciliata* Forsk. and *Acalypha malabarica* M. Arg. (Euphorbiaceae), University Campus, 10–8–55, P.N. Rao, O.U.B. Herb. 'Hy' No. 71.

11. *Oidium chrysanthemi* Rabh. Saccardo, P.A., **14** : 104. 1899.

Patel, M. K., Kamat, M. N. and Padhye, Y. A., *Indian Phytopath*, **2** : 142–155, 1944.

HABIT. On the living leaves of *Chrysanthemum indicum* L. (Compositae), University Campus, 30–11–55, P. N. Rao. O.U.B. Herb. 'Hy' No. 72.

12. *Oidium lagasceae* Chiddarwar.

Chiddarwar, P.P., *Lloydia*, **18** : 46–47, 1955.

HABIT. On the living leaves of *Lagasca mollis* Cav. (Compositae), University Campus, 6–11–55, P.N. Rao. O.U.B. Herb. 'Hy' No. 73.

*13. *Oidium* sp.

Mycelium superficial, creeping, hyaline, septate, branched, measuring 4.8 – 8.0μ in diameter, forming white coating only on the upper surface of the leaves. Conidiophores erect,2 – 3 septate, indistinctly. Conidia 1-celled, hyaline, oval to elliptic, simple with rounded ends measuring 27.2 – 35.0 x 15.2 – 19.2 μ.

HABIT. On the living leaves of *Cassia fistula* L. M. A. Salam. Narsapur Forest, 6–11–55. O.U.B. Herb. 'Hy' No. 74.

*14. *Oidium* sp.

Mycelium superficial, creeping, sub-hyaline, branched, septate, measuring 3.2 – 6.4μ in diameter, forming cottony white coating on both surfaces of the leaves. Conidiophores hyaline, straight, 2 – 3 septate, bearing 2 – 3 conidia in a chain. Conidia 1-celled, elliptical, measuring 30.4 – 51.2 x 12.8 – 16.0μ.

HABIT. On the living leaves of *Cassia tora* L. (Caesalpinaceae University Campus, 5–11–55, P.N. Rao. O.U.B. Herb. 'Hy' No. 75.

15. *Oidium erysiphoides* Fr.

Saccardo, P.A., *Syll. Fung.* **4** : 41, 1886.

HABIT. On the living leaves of *Bidens pilosa* L. (Compositae), University Campus, 6–11–58. P.N. Rao, O.U.B. Herb. 'Hy' No. 76.

*16. *Oidium* sp.

Mycelium cottony white, forming white patches sparsely on the upper surface of the leaves, creeping, hyaline, septate, branched, knobbed, measuring 3.6 – 7.2μ in diameter. Conidiophores straight, hyaline, 2-septate. bearing 2–6 conidia in a chain. Conidia hyaline, 1-celled, oval or elliptical, measuring 28.8–48.0 x 16.0 – 17.6μ.

HABIT. On the living leaves of *Datura fastuosa* L. Var alba cl. (Solanaceae), University Campus, 25–6–56, P. N. Rao, O. U. B. Herb. 'Hy' No. 77.

ACKNOWLEDGMENTS. The authors wish to express their indebtedness to Prof. M. Sayeeduddin, Head, Department of Botany and Principal, University College of Science, Osmania University, for his encouragement and keen interest in the work.

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KOORCHALOMELLA: A NEW GENUS OF TUBERCULARIACEAE

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(Accepted for publication October 15, 1958)

Among some of the recent collections of fungi made at Delhi, a bright orange coloured sporodochial fungus was found growing saprophytically on rotting paddy straw in a moist place. A description of the fungus is given below.

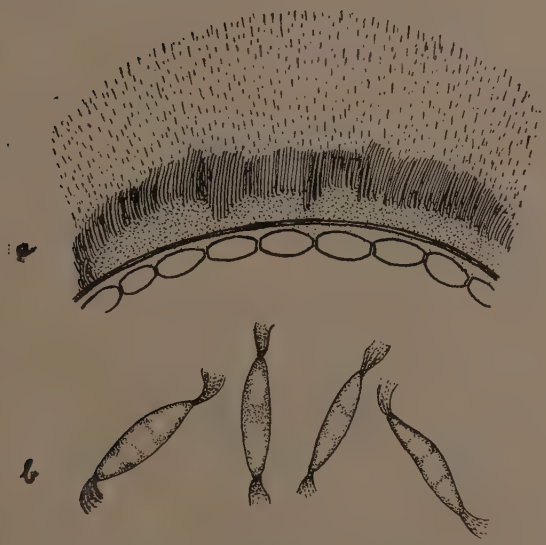
The *sporodochia* are scattered , separate or rarely confluent and circular to oval in outline. Occasionally a few become elliptic. They are superficial, bright orange to pinkish in colour with a white fringe. The *conidiophores* arise from a substratum of loosely interwoven hyphae and are simple, hyaline, cylindrical and compactly arranged to form a hymenium. The *conidia* are borne acrogenously and singly at the tips of conidiophores. They are hyaline, navicular to fusiform in shape, one-celled, with 1–2 (rarely 3-4) large vacuoules in the centre. The conidium, at each end, is provided with a single membranous, funnel-shaped or brush-like obconical appendage which is indistinct being hyaline but becomes clearly visible when stained with dilute aquous crystal-violet.

Subramanian (1953) erected a new genus *Koorchaloma* to accomodate a fungus possessing one-celled conidium with the peculiar brush-like apical appendage and the bright coloured setose sporodochia. Later in 1956, Agnihotrudu described another new genus *Starkeyomyces* characterized by *Koorchaloma* type of conidium with membranous appendage but not having setae in the sporodochium. In the same year, Subramanian described one more genus *Lomachashaka*, differing from the other two in having sporodochia fringed with numerous simple hyaline, sterile hairs. The fungus reported here differs from *Koorchaloma* Subr. and *Lomachashaka* Subr. in the absence of dark setae or hyaline hairs in the sporodochium, and from *Starkeyomyces* Agnih. in having conidia with brush-like membranous appendage at both ends. We have not come across any genus, which possesses conidia with brush-like appendage at each end, which are produced in bright coloured sporodochia devoid of setae or hairs and to accomodate this fungus, it is proposed to erect a new genus. Because of its affinities with *Koorchaloma* it is named as *Koorchalomella.*

Koorchalomella gen. nov.

Pertinet ad Fungos Imperfectos, ad Tuberculariaceas, Hyalosporas. Sporodochia lucide colorata, superficialia, integra. Conidiophori hyalini, simplices, cylindrici, efformantes seriem compactum hymenialem. Conidia hyalina, guttulata, continua, acrogena, appendicae membrancea ad utrumque apicem ornata.

Microphotograph showing conidia



a. Diagrammatic sketch of the Sporodochium.
b. Conidia.

Koorchalomella oryzae spec. nov.

Sporodochia dispersa, separata, raro confluentia, circularia vel ovalia ambitu; nonnumquam aliqua evadunt elliptica. Sunt vero superficialia et lucide aurantiaca vel rosea, margine albo. Sunt quoque magnitudinis variae, 1/3 ad 2 mm diam., 100 – 150 μ alta. Conidiophori emergunt e substrato constanti e hyphis laxe intertextis, suntque simplices, hyalini, cylindrici, arcte dispositi ad efformandum hymenium, magnit. 25–34 x 2–2.5 μ. Conidia producta acrogene et singulariter, hyalina, guttulis 4 – 6 ornata, semel cellulata, navicularia vel fusiformia, magnit. 15–20 x 2.5–3 μ. Ad utrumque apicem conidium ornatur appendice obconica unica penicillata magnitudinis 4.5 μ longit., 3 –7.5 μ latit. ad apicem latiorem.

Typus lectus in culmi emortuis *Oryza sativa* Linn., in loc. area Mycologica, I.A.R.I., New Delhi, 10.8. 1956, legit R. L. Munjal and J. N. Kapoor, et Positus Herb: Crypt. Ind. Orient numero 25558.

Koorchalomella gen. nov.

Fungus Imperfectus, Tuberculariaceae, Hyalosporae. *Sporodochia* bright coloured, superficial, entire. *Conidiophores* hyaline, simple cylindrical forming a compact hymenial layer. Conidia hyaline, guttulate, continuous, acrogenous, with a membranous appendage at both ends.

Koorchalomella oryzae sp. nov.

The *Sporodochia* scattered, separate (rarely confluent), circular or oval, occasionally a few becoming elliptic, superficial, bright orange to pinkish in colour with a white fringe; very variable in size ranging from 1/3 to 2 mm. in diameter and 100 to 150 μ high. *Conidiophores* arising from a substratum of loosely interwoven hyphae, simple, hyaline, cylindrical and compactly arranged to form a hymenium and measure 25–34 x 2–2.5μ. *Conidia* produced acrogenously and singly, hyaline with 1–2 (rarely 3–4) guttules, one-celled, navicular to fusiform in shape and measure 15–20 x 2.5 – 3μ. *Conidium*, at each end, provided with single brush-like, obconical appendage, 4.5μ in length and 3 – 7.5 μ in breadth at the broadest.

On dead culms of *Oryza sativa* Linn., Mycological area, I.A.R.I., New Delhi, 10.8.1956, coll. R. L. Munjal and J. N. Kapoor, Herb. Crypt. Ind. Orient No. 25558.

We are grateful to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology for helpful criticism and encouragement. Our thanks are also due to Dr. C. V. Subramanian for the loan of lectotype preparation of *Koorchaloma* and to Rev. Fr. Dr. H. Santapau for rendering the latin diagnosis.

Agnihotrudu, V. (1956) *J. Ind. Bot. Soc.* **35** (1); pp. 66–68.

Subramanian, C. V. (1953) *J. Ind. Bot. Soc.* **32** (3); pp. 123–126.

.....(1956) *Ibid*, **35** (1); pp. 38–42.

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STUDIES ON VIRUS DISEASES OF PLANTS IN MADHYA PRADESH
II. A NEW STRAIN OF DATURA VIRUS 3.

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(Accepted for publication October 15, 1958)

During the month of April 1957, a number of plants of *Datura innoxia* Mill. in the garden of Botany Department of Holkar College, Indore, were found showing severe disease symptoms. Older leaves near the base of the stem showed yellowing of the veins, very slight rolling of the margins inwards and reduction in size without any marked distortion of the leaf. Comparatively younger leaves show a marked reduction of lamina, puckering of the leaf surface and rolling of the lamina inwards. The normal dark green colour had disappeared from certain portions of the lamina. Mostly the raised portions of the lamina retained the dark green colour while the intervening portions were yellowish green. The youngest leaves showed extreme reduction, the lamina being just represented by the midrib only and being indistinguishable from the petiole, giving the leaf a thread like appearance. The colour of these leaves was yellowish green.

Six young healthy *Datura innoxia* plants, raised from seeds under insect proof conditions, were inoculated with the sap extracted from one of the above mentioned diseased *Datura* plants using carborundum powder as an abrasive. After a week symptoms started developing in each of the inoculated plants. The young newly developed leaves showed clearing of veins and slight rolling of lamina inwards starting near the base (which later on was found to be very characteristic of the disease). This was followed by a wrinkling of the leaf surface and loss of dark green colour from certain portions of the lamina. Mostly the raised portions of the lamina retained the dark green colour which disappeared from the rest of the lamina. Subsequently developed leaves showed a great reduction of lamina. The rolling of the lamina was very marked and in some cases such leaves were completely closed (as seen in figure 1) and the dorsal surface of the lamina was not visible. The reduction of the lamina appeared to start from the apex because there were certain leaves in which the apical portion of the lamina was thread like while the basal portion was fairly broad as is clear from figure 1. Leaves that were developed later were filiform and showed extreme reduction of lamina which was represented by the midrib only. This was very characteristic symptom and was present in all cases. The stem showed no abnormality. The infected plants produced flowers and fruits also, but the fruits produced by these plants were much smaller than those produced by healthy ones and were partially or wholly devoid of spines (figure 2) which are so characteristic of healthy fruits. None of the infected plant was killed by disease.

Transmission tests by mechanical inoculation using carborundum powder as an abrasive was repeated at different periods during the course

of an year. Every time the disease was easily transmitted by this method, However, weather conditions appeared to influence clearly the period after which the first symptoms appeared in an inoculated plant. During the summer months (April to July) the first symptoms of the disease appeared almost regularly a week after inoculation and almost all the inoculated plants got infected but during rainy season and winter months (August to February) the inoculated plants developed first symptoms of the disease two to three weeks after inoculation and percentage of infection was also comparatively low.

SEED TRANSMISSION. A large number of seeds collected from severely infected *Datura* plants were sown in pots from time to time with a view to see if the disease was transmitted through seed. Out of about three hundred plants thus raised none developed the disease which indicates that it is not transmitted through the seed.

HOST RANGE OF THE VIRUS. With a view to determine the host range of the virus, plants belonging to different families were tested at different periods of the year by means of mechanical inoculation. These included *Nicotiana tabacum* L., *Lycopersicon esculentum* Mill., *Solanum nigrum* L., *Solanum melongena* L., *Capsicum annuum* L., *Solanum tuberosum* L., *Hyoscyamus niger* L., *Petunia sp.* Garden variety, *Abelmoschus esculentus* Moench, *Luffa acutangula* Roxb., *Cucumis sativus* L., *Brassica rapa* L., *Daucus carota* L. and *Carica papaya* L.

It was found as a result of the above tests that the virus under investigation has an extremely limited host range and produced disease symptoms only on *Petunia*. About a week after inoculation, distinct vein clearing developed in the case of youngest leaves. This was followed by a marked wrinkling of the lamina and loss of dark green colour from certain portions of the lamina. These symptoms later on became more pronounced. Back inoculations on healthy *Datura innoxia* plants from *Petunia* produced typical symptoms of the disease.

PROPERTIES OF THE VIRUS. The Virus tolerates dilution upto 1 : 50 but not 1 : 100, exposure to 50 °C but not 55 °C for 10 minutes, and storage at 24 °C to 27 °C for 4 to 5 days.

DISCUSSION. Capoor and Verma[1] described the distortion mosaic virus of *Datura innoxia* Mill. from Bombay state and designated it as *Datura Virus* 3. The symptoms produced on *Datura innoxia* by the virus described herein resemble to a very great extent those produced by *Datura Virus* 3 on this plant, but differ in partial or total supression of spines on the fruit. Also, *Datura Virus* 3 has a higher thermal death point (62 °C.), a much higher dilution end point (above 1 in 10,000) and retains infectivity for longer period (13 days). It infects *Nicotiana tabacum* and *Lycopersicon esculentum* while the virus described in this paper does not infect these hosts. It is therefore proposed that the virus described herein be designated as *Datura Virus 3A* according to the classification followed by Smith [2].

1. Capoor, S. P. & Verma, P.M. (1952). Ind. J. Agric. Sci. 22 : 303—314.

2. Smith, K. M. (1937). A Text Book of Plant Virus Diseases. J. & A. Churchill Ltd. London.

ACKNOWLEDGMENTS. I am thankful to Shri D. W. Kshirsagar, Head of Botany Department, Holkar College, Indore, for providing all possible facilities and to Shri J. L. Merh, Plant Pathologist, Institute of Plant Industry, Indore, for allowing the use of his glass house and for providing other facilities. I also express my thanks to Shri P. R. Victor, Lab. Assistant in our Department who assisted me in the experimental work.

Fig 1

Infected leaves of *Datura innoxia*.

a a b

Fig. 2

Fruits on healthy (a) and infected (b) plants of *Datura innoxia*.

SUMMARY

A virus disease of *Datura innoxia* Mill. characterised by extreme reduction of leaf lamina, production of filiform leaves, and partial or total suppression of spines on the fruits has been described. The disease is easily transmissible by mechanical inoculation, but not through seed. The virus has a very limited host range and has been transmitted to *Petunia* sp. only in addition to *Datura*.

The virus has a dilution end point of 1 : 100, thermal inactivation point of 55°C. and logevity *in vitro* of 4 to 5 days at 24–27°C.

It is proposed to designate the virus as *Datura Virus* 3*A*.

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REACTION OF *ABELMOSCHUS* AND *HIBISCUS* SPECIES TO 'YELLOW VEIN' MOSAIC VIRUS

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(Accepted for publication November 1, 1958)

'Yellow vein' mosaic is a very serious disease of *Bhindi* (*Abelmoschus esculentus* (L) Moench) in India. It causes severe losses in yield and seriously mars the quality of fruit which is a popular vegetable. The disease was first reported by Uppal *et al* (1940) from the Bombay Province. Capoor and Varma (1950) described its symptomatology, and studied its host range. They also established *Bemisia tabaci* Gen. as its vector and Varma later (1952, 1955) studied the virus-vector relationship. Recently the disease has also been reported from Bihar and West Bengal (Jha and Mishra, 1955; Verma and Mukherji, 1955).

The main symptom of the disease in *Bhindi* is the vein clearing followed by veinal chlorosis of the leaves. The yellow net-work of veins is very conspicuous and the veins and veinlets are thickened (figs. 1 and 2). In severe cases the chlorosis may extend to the interveinal areas and may result in complete yellowing of the leaves. The fruits are dwarfed, malformed and are yellowish green in colour and their market value is reduced.

A survey of over 100 cultivated varieties and crosses of *Bhindi* grown in the Botany Division of the I.A.R.I. was made in 1952 but all were found to be diseased. The present investigations were, therefore, undertaken to test different species of *Abelmoschus* and *Hibiscus* for their reaction to the yellow vein mosaic virus in an attempt to search for suitable breeding material to evolve resistant varieties. The results of such tests are presented in this paper.

Material and Methods. The culture of the virus was obtained from a severely affected *Bhindi* plant in the field and was maintained on *Bhindi* plants in the glass-house by frequent transfers throughout the course of these investigations. Healthy plants of different *Abelmoschus* and *Hibiscus* species were raised from seed in the insect-proof glass-house and tested for their resistance to the 'yellow vein' mosaic virus by grafting and by feeding viruliferous white flies (*Bemisia tabaci* Gen.) in micro-cages. White flies were colonised on healthy tobacco plants in the insectary. About 10–15 white flies were rendered viruliferous by feeding them for 24 hours on a diseased *Bhindi* plant and liberated on each test plant. The test plants which were grafted or fed upon with viruliferous white flies and did not show any visible symptoms of disease were tested for the presence of the virus by feeding virus-free white flies on them and inoculating healthy *Bhindi* plants and were also tested by grafting on *Bhindi*.

EXPERIMENTAL RESULTS. Eight species of *Abelmoschus* and four of *Hibiscus* tested for their reaction to the virus were as follows:

Abelmoschus esculentus (L) Moench, *A. moschatus* Moench, *A. ficulneus* (L) Wt. & Avn., *A. tuberculatus* Pal et Singh, *A. manihot* (L) Medik, *A. manihot* (L) Medik var. *pungens* (Roxb) Hoch, *A. angulosus* Wt. & Avn., *A. crinitus* Wall. *Hibiscus cannabinus* L., *H. sabdariffa* L., *H. vitifolius* L. and *H. panduraeformis* Burm.

The results of the above tests are presented in Table I.

TABLE I. Results of inoculation of different species of *Abelmoschus* and *Hibiscus* with Yellow Vein Mosaic Virus.

Plant Species	Inoculation by grafting		Inoculation by feeding viruliferous white flies	
	No. of Plants		No. of plants	
	Inoculated	Infected	Inoculated	Infected
Abelmoschus esculentus	4	4	4	4
A. moschatus	4	4	8	5
A. ficulneus	6	3	2	2
A. tuberculatus	6	3	8	8
A. manihot	7	7	17	13
A. manihot var. *pungens*	6	0	11	0
A. angulosus	2	2	10	7
A. crinitus	2	0	10	0
Hibiscus cannabinus	4	3	6	3
H. sabdariffa	6	3	13	0
H. vitifolius	6	0	14	0
H. panduraeformis	6	0	6	0

The results of inoculation showed that *Abelmoschus esculentus, A. moschatus, A. manihot, A. ficulneus, A. tuberculatus, A. angulosus* and *Hibiscus cannabinus* could be infected by grafting as well as by feeding viruliferous white flies. *H. sabdariffa* was infected by grafting only. But *A. manihot* var. *pungens, A. crinitus, H. vitifolius* and *H. panduraeformis* could not be infected by either method. The healthy shoots from the inoculated plants of these species when grafted on healthy *Bhindi* did not produce any infections nor could the virus be recovered from them when white flies fed on these were liberated on healthy *Bhindi* plants. This indicated that they were immune to infection with the virus. The species of *Abelmoschus* and *Hibiscus* which were infected with the virus, however, showed great variation in symptoms ranging from yellow veinal mosaic to absence of veinal chlorosis and the presence of dark green vein swellings on the undersurface of the leaves. The characteristic symptoms produced on individual hosts are briefly described below:

Abelmoschus esculentus. The leaves show typical yellow vein mosaic symptoms and complete yellowing of the younger leaves. The vein swell-

ings are prominent on the undersurface of the leaves (Fig. 2). There is no appreciable reduction in leaf size.

Abelmoschus moschatus. The leaves show prominent bright yellow-vein mosaic symptoms and typical vein swellings on the undersurface of the leaves similar to those observed on *Bhindi* (Fig. 3).

Abelmoschus ficulneus. The leaves show vein clearing followed by veinal mosaic with dark green islands surrounded by yellow veins (Fig. 4). The leaves are slightly reduced in size. The margins of the leaves are curved downwards and the veins on the undersurface are thickened. Very few vein swellings are noticed on the older leaves only.

Abelmoschus manihot. The plants do not show any veinal mosaic of the leaves as is common on *Bhindi*. The leaves are of normal green colour and the plants may go undetected as healthy ones if not carefully observed. On close observation against bright light, the veins on the under surface of the leaves show minute dark green swellings at several places (Fig. 5). These may sometimes be absent on very young leaves. Shoots from such plants when grafted on *Bhindi* produced characteristic yellow vein mosaic symptoms on the latter (Fig. 6).

Abelmoschus tuberculatus. The leaves are very much reduced in size and become slightly curled and puckered. The petiole is bent and the leaves also tend to bend downwards. Typical yellow vein mosaic symptoms are not produced on the leaves, but the veins on the undersurface become more pronounced and numerous vein swellings are produced on them (Fig. 5).

Abelmoschus angulosus. The leaves are slightly reduced in size and show vein swellings on the undersurface but do not show the characteristic yellow vein mosaic symptoms commonly observed on *Bhindi* (Fig. 7).

Hibiscus cannabinus. The leaves show slight curling of the margins upwards giving it a cup shaped appearance. The undersurface shows vein thickening and vein swellings (Fig. 8). Later on the plants show recovery and the new growth does not exhibit any symptoms.

Hibiscus sabdariffa. The leaves of the infected plants show only vein swellings on the undersurface, otherwise they have a normal green colour and may go undetected if not closely observed (Fig. 5).

Discussion. Of the eight *Abelmoschus* and four *Hibiscus* species tested for their reaction to the *Bhindi* yellow vein mosaic virus, *Abelmoschus manihot* var. *pungens*, *A. crinitus*, *Hibiscus vitifolius* and *Hibiscus panduraeformis* proved to be immune to infection, as no transmission of the virus to these plant species by either method i.e. grafting or by insect agency could be obtained, and when indexed on *Bhindi* they did not show the presence of the virus in them. The remaining species tested although found to be susceptible showed great variation in the symptom picture ranging from typical yellow vein mosaic symptoms to mild forms which showed the absence of veinal chlorosis but the presence of only vein swellings.

The species of *Abelmoschus* and *Hibiscus* tested could be conveniently classified into three groups on the basis of their reaction to the *Bhindi* yellow vein mosaic virus.

1. Those which produce typical yellow vein mosaic symptoms such as *Abelmoschus esculentus, A. moschatus* and *A. ficulneus.*

2. Those which do not produce yellow vein mosaic symptoms but are characterised by the presence of vein swellings on the under-surface of leaves. These would include *Abelmoschus manihot, A. tuberculatus, A. angulosus, Hibiscus cannabinus* and *H. sabdariffa.*

3. Those which could not be infected with the virus and could be termed as immune to infection are *Abelmoschus manihot* var. *pungens, A. crinitus, Hibiscus vitifolius* and *H. panduraeformis.*

Summary

Eight species of *Abelmoschus* and four of *Hibiscus* were tested for their reaction to the yellow vein mosaic virus by grafting as well as by feeding viruliferous white flies (*Bemisia tabaci* Gen.). The symptoms produced on these hosts have been described. *Abelmoschus manihot* var. *pungens, A. crinitus, Hibiscus vitifolius* and *H. panduraeformis* have been found to be immune to the *Bhindi* yellow vein mosaic virus.

Acknowledgments. The authors are extremely grateful to Dr. R. S. Vasudeva, Head of the Division of Mycology & Plant Pathology for his keen interest, helpful suggestions and encouragement during the above studies and for kindly going through the manuscript. Thanks are also due to Dr. S. M. Sikka, Head of the Division of Botany for supplying the seed of *Abelmoschus* and *Hibiscus* species.

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Fig. 1. *Bhindi* plant affected with yellow vein mosaic virus.

Fig. 2. Upper (Left) and lower (Right) surfaces of the *Bhindi* leaves showing veinal chlorosis and vein thickening respectively.

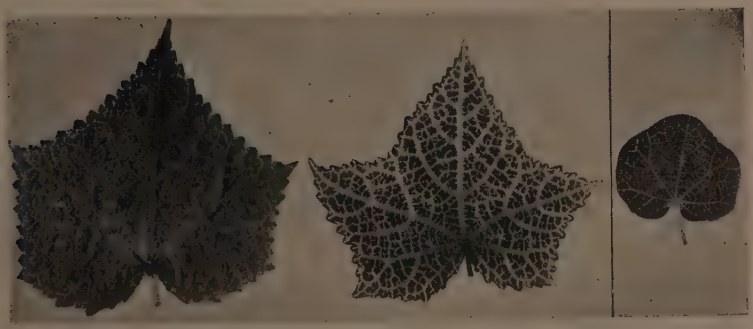

Fig. 3 Fig. 4

Fig. 3. Lower (left) and upper (right) surfaces of the leaves of *Abelmoschus moschatus* showing vein swelling and veinal chlorosis respectively.

Fig. 4. Leaf of *Abelmoschus ficulneus* showing yellow vein mosaic symptoms.

Fig. 5. Lower surface of the leaves of *Hibiscus sabdariffa* (left), *Abelmoschus tuberculatus* (middle) and *A. manihot* (right) showing vein swellings.

Fig. 6. Graft of *Abelmoschus manihot* (infected) on *Bhindi* showing the recovery of *Bhindi* virus from it.

Fig. 7

Fig. 7. Under surface of the leaf of infected *Abelmoschus angulosus* showing vein swellings.

Fig. 8

Fig. 8. Healthy (left) and diseased leaves of *Hibiscus cannabinus* showing vein swellings on the undersurface.

SALTATION IN *HELMINTHOSPORIUM ORYZAE* BREDA DE HAAN

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(Accepted for publication November 15, 1958)

Saltation in *Helminthosporium oryzae* Breda de Haan (*Cochliobolus miyabeanus* (Ito & Kuribay.) Drechsler ex Dastur) was reported by Matsuura (1930) who observed the fungus to saltate readily to produce a form with white patches of mycelium which remains white at 28 °C, but readily reverts to a dark colour below or above that temperature. The frequency of appearance of saltation, has been found to be conditioned by temperature and medium (Matsuura, 1930). Mitra (1931) also noted that in *Helminthosporium oryzae*, white saltant areas sometimes appear in culture. He did not however state the physiological conditions under which the saltant areas appeared in culture.

In course of cultural study of different isolations of *Helminthosporium oryzae* it was noted that white saltant areas appear rather frequently in culture under certain conditions. In view of the fact that knowledge on this subject is very limited, the behaviour of the isolates with reference to appearance of white patches or saltant areas was studied in detail.

Observations were made on twenty-three distinct isolates of *Helminthosporium oryzae* (isolated from paddy growing in different parts of West Bengal). The isolates were maintained in the laboratory on P.D.A. at 20 °C. For purpose of study of saltation they were grown on three different media, namely maize meal agar, oatmeal agar and Richard's agar in Petridishes, each Petridish containing 20 c.c. of the medium. The Petridishes were inoculated at the centre with the bits of mycelium from seven days' old culture maintained in potato dextrose agar at 20 °C and were kept at four different temperature, namely 21 °C, 26 °C, 31 °C and 36 °C. A replication of five Petridishes was used for recording observations.

The isolates in different culture media at 20 °C–21 °C usually form a rich felty or matted dark greyish or blackish growth occasionally showing faint zonation. Under certain conditions, (stated later) mycelial patches appear during the course of growth, which differ strikingly from the rest, being white in appearance in contrast to the usual dark greyish colour of the mycelium. These white patches of mycelial growth constitute the "saltant" areas, as shown by Matsuura (1930).

Considerable variation was observed among the different isolates in the appearance of these white patches, their frequency and manner of distribution in the Petridishes. On the basis of size, manner of appearance and distribution of the "saltant" areas in the isolates at 26 °C, the isolates may be put under four different groups as detailed below:—

Group One. The mycelial growth shows distinct zonation of alternate layers of raised and depressed mycelial growth. The white mycelial areas appear on the ridges as small raised, spherical, ball-like bodies measuring 2–12 mm. in diameter, those near the centre being larger than those at the margin. These areas are sharply defined from the normal mycelial growth and are composed of very densely crowded hyphae which make these areas very hard and compact. These areas appear in large numbers and often coalesce with each other, but in no case the zonate character of the mycelial growth is marked (Fig. 1). This type of growth has been found to be characteristic of five isolates.

Group Two. The mycelial growth, as in the first case, shows distinct zonation of alternate layers of ridges and furrows. In the ridges, the mycelial growth is very light purple to whitish, in colour in contrast to the dark grey colour of the mycelium in the furrows. On the ridges or raised areas, white compact mycelial areas or saltant patches appear (Fig. 2). Very often fanshaped sectors also appear in the culture media. The sectors are light grey in colour and are composed of greyish hyphae without any saltations. (Fig. 3).

In some cases the saltant areas enlarge, coalesce with each other and partially cover the mycelial growth in the plate (Fig. 4). These white patches are not sharply defined as in Group one. This type of growth has been noted in ten isolates.

Group Three. The mycelial growth is faintly zonate and the white mycelial areas appear in irregular fashion. When fully developed, these areas are compact, spherical, large, measuring 5–18 mm. in diameter. These areas may or may not be arranged in a concentric manner. These areas which are at first very sharply defined from the rest of the dark mycelial growth, often coalesce at maturity. It is not unusual to obtain a whitish growth covering the entire plate (Fig. 5 and 6). This type of growth has been observed in seven isolates.

Group Four. Comprising of one isolate only is characterized by the appearance of a white appressed patch, may be two or a few at the centre in the Petridishes. The mycelial growth does not show any zonation. The patches are always restricted to the centre of the growth and are circular to irregular shaped and attain a diameter of 5–25 mm. (Fig. 7).

It may be stated that these white 'saltant' areas appear in the Petridishes during the course of the growth of the mycelium in the Petridishes. These areas begin to appear after 3–5 days of growth after the inoculation of the Petridishes.

Effect of Temperature. Regarding the effect of temperature on the appearance of these whitish 'saltant' areas in the culture, it may be stated that the characteristic type of growth of each group is observed best at 26°C which was found to be most favourable for the purpose. At a higher temperature of 31°C appearance of these areas is irregular. The 'saltant' areas are not sharply defined, and they are flat and depressed

instead of being spherical ones (Fig. 8 - showing the saltation at 31 °C of an isolate in Group One). In these patches, parental hyphae are also occasionally present. Arrangement of saltant areas in zones on the ridges, as noticed in many cases at 26 °C is not clearly evident. At 36 °C, saltation is practically absent. The 'saltant' areas wherever they appear are irregular, ill-defined, scattered without any definite arrangement and mixed with parental hyphae. At 21 °C, saltation is practically absent and the saltant areas, appear in very few numbers only in maizemeal and oatmeal agar and sparingly in Richard's agar or may not be formed at all.

Effect of Medium. At 26 °C, which is the optimum temperature for the saltation, the appearance of the 'saltant' areas has been found to be more pronounced in maizemeal agar and oatmeal agar than in Richard's agar. In Richard's agar, the 'saltant' areas are not compact, but they are of loose texture and wooly in appearance. They are also comparatively less sharply defined.

Conidial Production. In the compact white 'saltant' areas, conidial production is practically absent, though the conidia may be freely and abundantly produced in the adjacent areas showing greyish mycelial growth. In saltant areas of loose texture, conidia are sparingly produced, and they are much smaller in size - 32.3 – 91.2 x 7.6 – 13.0 μ with 7 septa, averaging 62.7 x 11.4 μ with 5 septa, as compared with typical conidia measuring 34.47 – 158.95 x 7.66 – 17.24 μ with upto 12 septa.

Reversible Nature of Saltant Area. Matsuura (1930) stated that the saltant areas revert to their dark colour on exposure to a temperature above or below 28 °C. In studies with saltations in this case, it has been observed that subculturing from purely white saltant areas results in gradual reversion to typical parental form in 4 – 5 generations of growth (each generation extending for a period of three weeks) at 21 °C. At 26 °C, reversion to parental form, is very slow. For three to four generations, the subculturing from a saltant area and subsequent growth at 26 °C results in white form which tends to come back to a greyish growth or a mixture of black and white with age, but the original parental greyish black mycelial growth is never attained in this case. When a mixture of greyish black and white mycelium is used as inoculum, reversion is quickly accomplished at 21 °C. The parental form, thus attained, when grown on the oatmeal or maizemeal media at 26 °C, again shows appearance characteristic of 'saltant' areas.

It may be worth-while to mention that the characteristic type of saltation produced by any isolate is constant for that isolate for any number of generations in different media and saltation in *Helminthosporium oryzae* may be considered to give a valuable aid in the study and distinction of cultural groups.

Summary

1. Study of twenty three isolates of *Helminthosporium oryzae* in West Bengal, reveals that 'saltation' is rather frequent in the fungus under certain conditions.

2. On the basis of size, manner of appearance and distribution of the saltant areas in the isolates at 26 °C, the isolates may be divided into four distinct groups.

3. Temperature of 26 °C was found to be most favourable for the appearance of the saltant areas. At 31 °C, saltation is irregular and saltant areas are ill-defined. At 36 °C saltation is practically absent and at 21 °C, saltation is rather infrequent.

4. Saltation is more pronounced in maizemeal agar and oatmeal agar than in Richard's agar.

5. Conidial production is practically absent in a typical 'saltant' area. Very few conidia are produced in a loose 'saltant' area and are smaller in size 32.3 – 91.2μ x 7.6 – 13.4μ with upto 7 septa (average size 62.7 x 11.4μ – 5 septa) as compared with the normal size 34.47 – 158.95 x 7.66 – 17.24μ with upto 12 septa.

6. Reversion of the 'saltant' growth to original parental dark grey mycelial form takes place gradually at 21 °C on repeated subculturing. But at 26 °C, complete reversion to parental form does not take place, even after a number of generations in subcultures.

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EXPLANATION OF FIGURES IN THE PLATE

Fig. 1: Group One. Culture showing sharply defined ball like saltant areas on the concentric zones at 26°C.

Fig. 2: Group Two. Showing alternate layers of ridges and furrows and whitish mycelial growth on the ridges in concentric manner and also compact saltant areas on the ridges at 26°C.

Fig. 3: Group Two. Showing the appearance of sectors within concentric saltant areas at 26°C.

Fig. 4: Group Two. Numerous small saltant areas on the ridges and furrows coalescing and partially covering the mycelial growth at 26°C.

Fig 5: Group Three. Heavy ball-like saltant areas almost covering the mycelial growth at 26°C.

Fig. 6: Group Three. Saltant areas have completely covered the mycelial growth at 26°C.

Fig. 7: Group Four. Showing one broad saltant area at the centre at 26°C.

Fig. 8: Group One. The saltant areas which were found to be compact and sharply defined at 26°C, are appearing as flat, ill defined and spreading in the Petridishes kept at 31°C.

ASSESSMENT OF TUBER TRANSMISSION OF LEAF – ROLL AND RUGOSE MOSAIC OF POTATO IN WEST BENGAL

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(Accepted for publication November 15, 1958)

Chattopadhayay and Das (1955) after a preliminary survey, stated that in West Bengal Leaf-roll and Rugose Mosaic are the two serious virus diseases of Potato. Potato viruses are in all cases tuber borne. It is an agreed fact that when a plant is grown from a virus infected tuber all the new tubers formed by it carry the virus. Various workers have also reported that a progeny of newly infected plant often consists of a mixture of healthy and diseased tubers (Doncaster and Gregory, 1948). Doncaster and Gregory (1948) have also shown that in a mixed progeny 50 to 60 per cent of the tubers were infected with Rugose Mosaic and Leaf-Roll. Pal (1943) observed that tubers from infected plants always do not give rise to diseased plants, usually 50 to 60 per cent of the resulting plants show disease. Mitra and Mehta (1950) have also reported that in a mixed sample the chances of mosaic infection in the tubers become less with increase in size of seed tubers. So a preliminary experiment was undertaken to determine to what extent the seed tubers collected from affected fields are infected with Rugose Mosiac and Leaf-Roll.

The potato tubers of the variety *Darjeeling Red Round* were collected irrespective of size from cultivators' plots from two localities - Dhamatikar and Noornagar - in lots of ten. They were collected separately from apparently healthy, Leaf-Roll affected, and Rugose Mosaic affected plants. For this purpose, the plants were marked during the growing season and the tubers were collected at the time of harvest in March. They were stored at a temperature of 65° to 70°F. and sown in the month of September in pots with a single tuber in each pot. The plants were grown and kept in insect-proof cages throughout the course of experiment. The symptoms were noted when the seedlings were one and half months old. The presence or absence of virus was confirmed by sap inoculation on differential hosts - *Datura stramonium*, tomato and tobacco.

When tubers collected from apparently healthy plants from Dhamatikar were sown, some of the plants coming up showed mild interveinal mottling. Sap transmission from such plants on *Datura stramonium* resulted in light and dark green mottling without necrosis and on tomato dark and light green mottling, which indicated the presence of *Potato virus-X* (*Solanum virus* 1 Orton) in the plants. On an average 54.5 per cent of the tubers carried this virus. A few plants of the same lot showed very faint mosaic symptoms as well as fine necrosis on the veins of the lower surface of the leaves, later on the necrosis passed down to the leaf petiole and in some cases on the main stem forming longitudinal brown streaks. The leaf margin became scortchy, ultimately withered and remained attached to the

blackened, withered and drooping petiole giving the appearance of attack of "Late blight". The symptoms observed in this case corresponded to that of *Potato virus Y* (*Solanum virus* 2 Orton)- negligible mosaic with veinal necrosis as described by Vasudeva and Lal (1945) on Phulwa variety. The presence of which in these plants was detected by sap inoculation on tomato and tobacco. On tomato mild symptoms of vein clearing and mottling were noticed at the begining which later developed into charateristic vein-banding symptoms. On tobacco it developed vein clearing after 12-15 days of inoculation. This vein clearing with age changed into vein-banding symptom. The symptoms produced on tobacco correspond no doubt to that produced by Potato virus A but in the absence of Potato virus A in the potato fields in West Bengal sap inoculation on differential hosts - *Solanum nodiflorum* and *Physalis floridana* was not done. 18.1 per cent of the tubers were found to carry this virus. Potato virus Y was first reported by Pal (1943) on potato variety Phulwa. Subsequently Vasudeva and Lal (1945) reported its occurrence on the same variety and made a detailed study.

The plants coming up from the tubers from Noornagar showed the symptoms of Rugose Mosaic only in 37.5 per cent cases.

In respect of the tubers collected from Dhamatikar and Noornagar localities from plants affected with Rugose Mosaic it was observed that the progenies coming out showed typical Rugose Mosaic symptoms in 55.5 and 40.0 per cent cases respectively. Plants which did not show any symptoms were tested on differential hosts for the presence of 'X' and 'Y' but none developed any symptoms proving thereby that though the plants were grown from tubers collected from Rugose Mosaic affected plants yet they have not carried any virus.

The plants that were grown from tubers collected from the above localities from Leaf-Roll infected plants showed 35.7 and 50.0 per cent Leaf-Roll symptoms. The young plants were slender and somewhat spindly with general pallor on the young leaves which was followed by slight rolling at the base of leaves.

The results point out that in an infected field the plants apparently showing no symptoms may also carry the virus; similarly the tubers collected from diseased plants may not necessarily carry the virus particularly in case of late season infection.

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"TARGET LEAF SPOT" DISEASE OF COFFEE. I—OCCURRENCE, SYMPTOMS AND ETIOLOGY.

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(Accepted for publication November 15, 1958)

A leaf spot disease of *Coffea arabica* was noticed at Coffee Research Station, Balehonnur (Mysore State), during the South West Monsoon, 1955. It was again observed in a few estates in and around Balehonnur and Sakleshpur (Mysore State), during August, 1957. The disease was found mostly on young nursery seedlings and to a lesser extent on mature coffee-particularly with dense growth. A preliminary survey of nursery seedlings, 6 to 8 months old, in one of the estates showed an incidence ranging from 5 to 33 per cent. In this nursery an appreciable degree of defoliation of affected leaves occurred. The disease has been observed to occur only on arabica coffee so far.

Symptoms. The disease is seen to occur mainly on the leaves (Fig. 2); and tender stems and fruits are rarely affected. The disease commences as minute roundish spots, oil soaked in appearance and about 1 mm. in diameter, turning brown later. Under favourable moisture and temperature conditions the spots enlarge often to 20 to 25 mm. in diameter, within 4 to 5 days. On the upper surface of these spots distinct concentric zonation is visible which presents a characteristic "target board" appearance (Fig. 3). Black fruiting bodies are noticed on the under surface of the affected leaf along these concentric rings. The fruiting bodies are superficial and blackish with white ciliate margins. (Fig. 4.) They are formed in profusion under favourable environmental conditions. Several diseased spots may occur on a leaf; they often run into each other to form large irregular discoloured patches. Such patches are invariably bound by a faint yellow halo. As the disease progresses, particularly during spells of dry weather the tissues dry up, become brittle and crumble. When the disease incidence is serious particularly on seedlings, defoliation of affected leaves follows. Fruiting bodies have also been observed in rare instances on mummified semi-ripe fruits.

Morphology. Fungal mycelium is noticed in the affected tissues. It is both inter and intra-cellular and is composed of slender hyaline hyphae ramifying the parenchymatous leaf tissues. Death of host cells in advance of the ramifying hyphae is noticed, indicating a possible secretion of some enzyme by the fungal mycelium which may cause the death of the cells.

The fruiting bodies (sporodochia) found on the diseased spots are hypophyllous, superficial, gregarious sometimes becoming confluent,discoid-scutellate occasionally appearing globose due only to the piling up of the conidial mass (Figs. 5 & 6), black with white ciliate margins and measuring 160 to 350μ at the broadest points. The sporodochia arise from a pseudoparenchymatous stroma and are composed of fertile

hyphae (conidiophores). The conidiophore is septate, hyaline, consisting of a main axis which is usually branched more than twice, and crowned with a whorl of closely appressed phialides (figs. I, 1 to 6 & Fig. 7), and measuring 115 to 165μ from the base to the top of the phialide layer. The unbranched lower portions of the numerous stipes are agglutinated together to form a closed synnema. From the stipes some of the branches arising laterally remain sterile giving rise to the white ciliate fringe of the sporodochia (fig. I, 2). The branched middle and top portions of the conidiophores, before they end in the phialides, form a lax or open weft (Figs. 5 & 6). The ultimate branches of the conidiophores measure 6.6 – 11.6 x 1.9 – 3.2μ and end in a cluster of narrowly clavate phialides (fig. I, 4 to 6). The phialides are hyaline at first and later turn darker. They measure 10.2 – 24 x 1.8–3μ, and form a closely appressed whorl, on which olivaceous black, glutinous masses of conidia are borne. The conidia are acrogenous, borne directly on the phialides (fig. I, 7), cylindrical, rounded at both ends, smooth walled, unicellular, usually 2 - but occasionally 3-guttulate (fig. I, 8), hyaline at first later becoming dark. They measure 5.6 – 7 x 1.4μ (5.8 x 1.4μ). The thick glutinous black masses of conidia form an olive green suspension in water.

When grown on potato-dextrose-agar, the fungus produces submerged mycelium at first,the surface growth being moist and much convoluted. Later, scant white aerial mycelium is produced. Within 3 to 4 days the black fruiting bodies which are gregarious and often confluent are produced.

Pathogenicity. Preliminary pathogenicity trials were conducted on *Coffea arabica*. Drops of spore suspension in sterile water prepared from a 19-day old culture of the fungus were inoculated on healthy leaves of 8-month old plants growing in pots. Seven pairs of leaves were inoculated at two points per leaf with an equal number as controls against each treatment. After inoculation the plants were covered with alkathene bags for 24 hrs. to provide a high humid condition. The results are given below:

Table 1. Results of preliminary infection trials with a 19-day old culture of the fungus on arabica coffee.

(Max. Min. temp. range during the expt. : 27.9–17.8°C)

Treatment	Leaf surface inoculated	No. of points inoculated	infected
1. Injury with pin pricks	Upper	28	28
	Lower	28	28
2. Without injury	Upper	28	24
	Lower	28	15

Spots began to appear on injured tissues within 48 hrs. and in the case of uninjured tissues within 72 hrs. Sporodochia appeared on the spots on the 8th day, forming mature conidia on the 9th day. The controls remained healthy throughout. Injury favoured but was not a

necessary prerequisite for the success of the infection. Infection occurred successfully irrespective of the leaf surface inoculated.

Reisolations from the resulting leaf spots yielded the same fungus as originally used for inoculation, indicating the fungus as the causal agent of the disease.

IDENTIFICATION. The fungus causing this disease has been identified as *Myrothecium advena* Sacc.

Saccardo[1] (1908) described a '*Myrothecium*' forming sporodochia on the under side of fading leaves of *Coffea arabica* in a hot house at Cherbourg (France) with the specific epithet '*advena*', in view of its adventitious appearance on this host. The present paper appears to be the first record of *Myrothecium advena* Sacc. as an active parasite of coffee. In view of the primary parasitic nature of this fungus, the specific epithet '*advena*' appears to be somewhat incongruous.

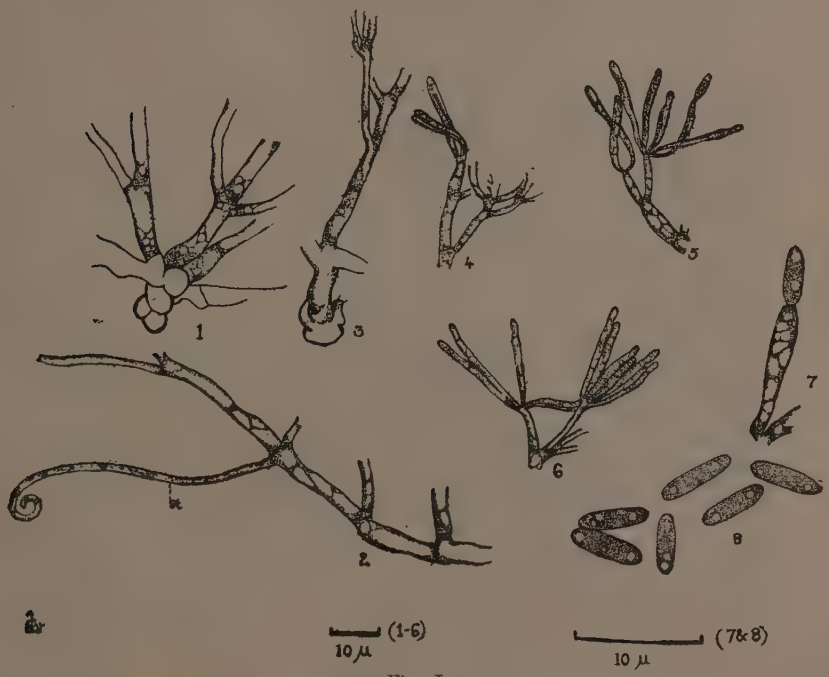


Fig. I

1–3. Conidiophores showing branching habit. st: sterile branch.
4–6. Cluster of phialides borne on the terminal branches of conidiophores.
7. Single phialide showing conidial attachment.
8. Conidia.

1. Saccardo (1908). Sylloge Fungorum 22 : 1493.

Fig. 2. *Coffea arabica* leaves showing affected spots.

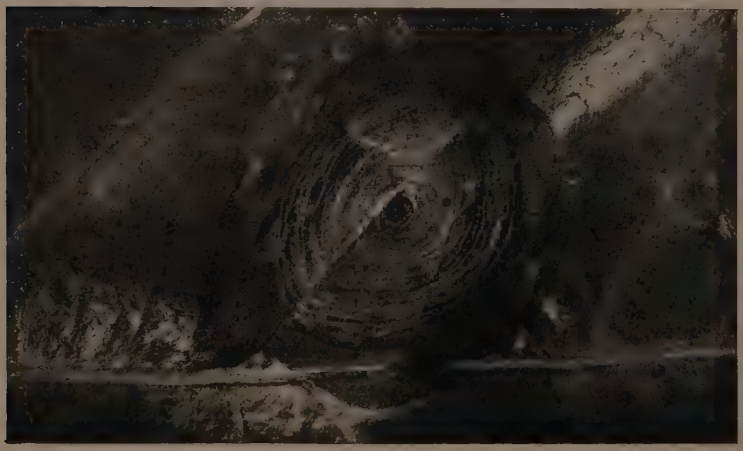


Fig. 3. Upper surface of the spot showing concentric zonations.

Infected leaves of *Coffea arabica* with the characteristic sporodochial formation have been collected and preserved as herbarium specimens at Coffee Research Station, Balehonnur. Herbarium specimens and a pure culture of the fungus (IMI 70817) have been deposited with the Commonwealth Mycological Institute, England.

STATUS OF THE DISEASE. Field observations have indicated that the disease has not acquired a major status so far. Investigations on the various aspects of the disease are in progress.

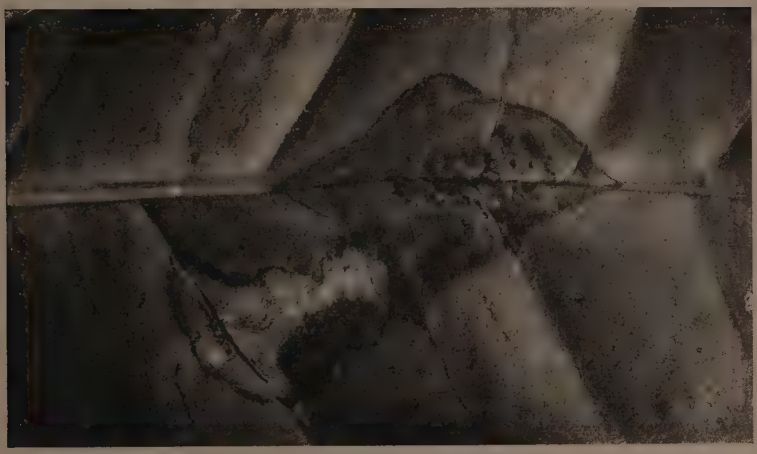
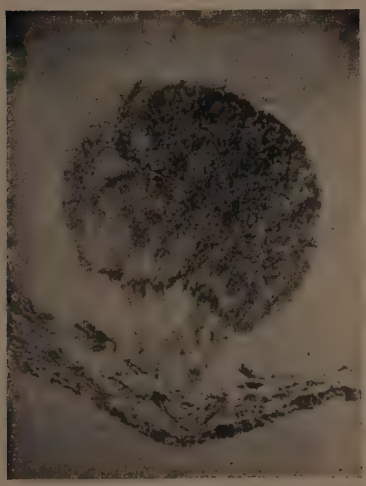


Fig. 4. Under surface of the spot showing blackish sporodochia with white margins.



5 6

Figs. 5 & 6. Globose and discoid sporodochia

Summary

A new leaf spot disease of *Coffea arabica* noticed in a few coffee plantations in and around Balehonnur and Sakleshpur areas of Mysore State, India is described. The disease is found mostly on young seedlings: it is also sometimes noticed on mature bushes with dense growth. The

Fig. 7. Branching conidiophores, with terminal branches ending in phialides

disease is seen mostly on leaves; rarely tender stems and fruits are affected. The disease spots exhibit a characteristic "target board" appearance on the upper surface. On the lower surface, superficial black sporodochia with white ciliate margins are seen. Severe infection causes defoliation of affected leaves. The morphology of the fungus is described. Preliminary pathogenicity trials showed that the fungus is an active parasite on *Coffea arabica*. The fungus is identified as *Myrothecium advena* Sacc. The disease has not acquired a major status in South India so far. Further work on the various aspects of the disease is in progress.

The writers acknowledge with grateful thanks the help rendered by the Director, Commonwealth Mycological Institute, England, for the specific identification of the fungus. Thanks are also due to Mr. E. W. Mason of the above Institute for kindly going through the manuscript and for his valuable suggestions. They are deeply indebted to Dr. B. T. Narayanan, Director of Research, Coffee Board, for his kind encouragement during these studies.

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SOME VIRUS DISEASES OF TEMPERATE FRUITS

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(Accepted for publication November 15, 1958)

Virus diseases of temperate fruits have received considerable attention in the Western countries, particularly in North America where more than 40 virus diseases are known to affect the stone-fruits alone (Cochran *et al*, 1953). In India, the investigations on virus diseases of temperate fruits were taken up in 1953 at the Simla Sub-station of Indian Agricultural Research Institute. The results of investigations carried out so far in this direction are briefly reported in this note.

The present area under temperate fruits in India has been estimated at 40,080 acres which is rapidly expanding under the current development plans resulting in considerable movement of plant material from one area to another within the country as well as importations from abroad. Virus diseases of these plants are reported to be responsible for heavy losses and their study, therefore, in any further horticultural development programme requires due consideration (Vasudeva, 1957; Azad and Sehgal, 1957).

A systematic and intensive survey of fruit orchards for virus diseases was undertaken in Simla Hills and Kulu Valley which are important areas for cultivation of temperate fruits. The occurrence of line-pattern and mosaic diseases of plum and mosaic of himalayan raspberry (*Rubus ellipticus* Smith) is fairly widespread. In addition, the presence of mosaic and ring-spot mottle in apple and a suspected mosaic in sweet cherry (*Prunus avium* L.) have also been observed.

Line-pattern of Plum. The disease which is apparently similar to the one described by Cation *et al* (1951) was first observed in plum trees growing in the vicinity of Simla Station. Transmission tests conducted during 1953-54 established its virus nature (Azad, 1958). The characteristic symptoms consist of yellow vein-banding in part or whole of the leaf lamina, with occasional development of oak-leaf patterns (Plate I, fig. 1). The disease has been successfully transmitted under controlled conditions by grafting to seedling peach (*Prunus persica* (L.) Batsch.) (Plate I, fig. 2), seedling almond (*P. amygdalus* Batsch.) (Plate I, fig. 3), and a common wild prune, *pajja* (*P. puddum* Roxb.), besides plum seedlings and plum varieties Formosa and Green. Its transmission was also indicated in Santarosa plum.

The occurrence of this disease in plum has also been reported from Kumaon (Bhargava and Bist, 1957a and 1957b) and Kalimpong areas in the mid-and-eastern himalayan regions.

Plum Mosaic. A disease of plum showing characteristic mosaic symptoms (Plate II, fig. 4) has been found to be widespread in different

localities of Simla Hills. The virus nature of the disease has been established in transmission tests conducted under controlled conditions. In host-range studies, the disease has so far been transmitted by grafting to seedling peach (*P. persica*) and *P. puddum* as also to plum varieties Green Gage, Becky Smith and Green, and Satsuma plum. Transmission was also indicated in plum var. Beauty. Colour tests for the detection of virus infections in diseased leaves after the technique of Linder *et al* (1950) applied with some modifications have shown the virus nature of the disease. Similarly, chemical tests designed for the detection of polyphenols accumulating in plant tissues (Reeves, 1951) have also given positive reaction.

Mosaic of Himalayan Raspberry. A severe disease characterised by yellow or light green mosaic, ring-spot mottle, and oak-leaf pattern (Plate II, fig. 5) has been observed in the himalayan raspberry (*Rubus ellipticus* Smith) which grows wild in Simla Hills. The incidence of the disease is above 50 per cent in most of the localities. In addition to the symptoms described, line-pattern and complete chlorosis and smalling of leaves have also been observed in some cases.

As the disease appears to be of complex nature, investigations were taken up, to start with, on a diseased plant showing typical mosaic symptoms. The disease has been successfully transmitted by inarch-grafting as well as by bottle-graft technique to *R. ellipticus* and *R. macilentus* Camb. Transmission of the disease to healthy *R. ellipticus* through dodder (*Cuscuta reflexa* Roxb.) has also been obtained; it appears to be the first record of transmission of a plant virus by this species of *Cuscuta*.

In transmission tests by grafting the component causing line-pattern symptoms on *R. ellipticus* has also been isolated from the mosaic complex, which is being further investigated.

Apple Mosaic. Two types of mosaic - one causing yellowish flecking and mottling of leaves and the other manifested by a diffused ring-spot mosaic mottle - have been observed in the apple orchards in Simla Hills. The first type (Plate II, fig. 6) which has been successfully transmitted by bud-grafting has been observed mainly in a newly introduced variety Red Gold, and apparently resembles the mild mosaic described by Posnette and Cropley (1952). The second type has been found widespread in some old plantations of Golden Delicious variety and indication has been obtained of its virus nature.

Disorder showing virus-like Symptoms in Cherry. In sweet cherry (*Prunus avium* L.) var. Black Heart some trees in Kulu Valley have been found affected with a variegated mosaic (Plate II, fig. 7). As the variety of cherry involved is most important from commercial point of view and also because the affected trees appear to be considerably reduced in vigour, the cause of the variegated mosaic is being investigated.

Along with the initiation of investigations on virus diseases of temperate fruits, a nursery of different varieties of healthy fruit trees which are important from commerical point of view as well as those which are used as differentials has been started at the Simla Station.

ACKNOWLEDGEMENT. The authors are greatly indebted to Dr. R. S. Vasudeva, Head of the Division of Mycology & Plant Pathology, I.A.R.I., New Delhi, for his valuable guidance and constant encouragement during the course of the investigations.

Thanks are also due to Mr. K. Kirpal Singh, Fruit Specialist, Punjab, and Mr. A. R. Thapar, Horticultural Officer, Himachal Pradesh, for their kind co-operation in providing facilities at their stations for observations.

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Fig. 1. Plum leaves showing range of symptoms of line pattern disease.

Fig. 2. Symptoms on peach leaves produced by the virus causing line pattern disease of plum.

Fig. 3. Symptoms on almond leaves produced by the virus causing line pattern disease of plum.

Fig. 4. Plum leaves showing range of symptoms of mosaic disease.

Fig. 6. Yellow flecking mottle of apple.

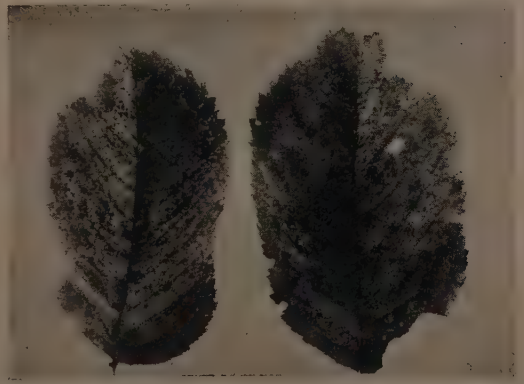

Fig. 7. Cherry leaves showing symptoms of variegated mosaic.

Fig. 5. Mosaic of himalayan raspberry (*Rubus ellipticus*).

STUDIES ON THE MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL PROPERTIES OF FIVE ANTIBIOTIC-PRODUCING ISOLATES OF THE *STREPTOMYCES LAVENDULAE* GROUP

G. Rangaswami

(Accepted for publication November 10, 1958)

The *Streptomyces lavendulae* group of organisms are widely distributed in the soil. They are characterized by their cream-coloured to brownish growth, with lavender or vinous lavender aerial mycelium, spiral sporophores, strong proteolytic and diastatic properties, and producing brown to black soluble pigment in protein-containing media (Waksman and Lechevalier 1953). Several isolates of this group have been reported to produce antibiotic substances, the most important ones being the strains of *S. lavendulae* (Waksman and Curtis) Waksman and Henrici producing streptothricin and related antibiotics (Waksman and Lechevalier) 1953), *S. venezuelae* Ehrlich *et al* producing chloromycetin (Ehrlich *et al* 1948), *S. virginiae* Grundy *et al* producing actithiazic acid (Grundy *et al* 1952) and isolate Nos. 3716 and 3717 producing mycothricin complexes A and B, respectively (Rangaswami *et al* 1956). With a view to compare the morphological, cultural and physiological properties of these organisms and to understand their nutritional requirements studies were undertaken at the Institute of Microbiology, Rutgers University, New Brunswick, New Jersey, U.S.A., during 1955-56 and the results are presented here.

Material and Methods *S. lavendulae* No. 3440–14, the strain known to produce streptothricin, was obtained from the Institute of Microbiology Culture Collection. *S. venezuelae* No. 3534 and *S. virginiae* No. 3651 used in these studies were obtained from Dr. David Gottlieb, Illinois University, Urbana, Ill. and Abbot Laboratories, North Chicago, Ill., U.S.A., respectively. The strains, No. 3716 and No. 3717, were isolated by the author from a soil sample collected near New Brunswick, New Jersey, U.S.A

Preparation of various media and the details of the tests carried out in these studies were essentially the same as described in the Supplement to the Bergey's Manual of Determinative Bacteriology (Anonymous 1953).

Morphological characters of the cultures were studied on agar slides. Melted agar media at 47 °C were mixed with spore suspension of the cultures and pipetted aseptically on sterile slides in moist chambers. The agar was spread on the slide to solidify into a thin film so as to facilitate direct examination under microscope. The cultures were incubated at 28 °C and examined periodically.

Utilization of various carbon and nitrogen sources by the cultures was studied by growing them under submerged aerated conditions in

Pridham and Gottlieb's basal medium (Pridham and Gottlieb 1948); 250 ml. Erlenmeyer flasks containing 100 ml. of the medium were used. One per cent of glucose or other carbon sources to supply equivalent amount of carbon and one per cent glutamic acid or other nitrogen sources to supply equivalent amount of nitrogen were added to the medium. The inoculum was obtained by growing the cultures in nutrient broth under submerged aerated conditions for 48 hours and washing in changes of saline. One ml. portions of the suspensions of the washed cells were used for inoculating each flask. After ten days' growth the mycelium was filtered through Whatman No. 1 filter paper and the dry weight obtained.

EXPERIMENTAL RESULTS *Morphological characters:* The morphological properties of the isolates 3716 and 3717 were compared with 3440-14 by growing them on agar slides in nutrient, yeast extract-glucose and Bennett's agar media. Varying degrees of sporulation of the three cultures were observed in the three media, but all of them produced abundant spores in Bennett's agar after seven days. The culture 3716 produced straight or narrow spiralled conidiophores, whereas 3717 and 3440-14 produced mostly straight conidiophores, spirals being observed only rarely. The diameter of the hyphae in all the cases measured 0.8 – 1.0μ. The spores of 3716 were spherical to oval measuring 0.6 – 1.0 X 0.6 – 0.8μ; those of 3717 oval to cylindrical with 0.8 – 1.4 X 0.8 – 1.0μ; those of 3440–14 were oval to oblong with 0.7 – 1.6 X 0.4 –0.8 μ.

Cultural Properties: A comparison of the cultural properties of the five isolates is reported in Table I. The isolate 3716 seems to come closer to 3440–14 in most cultural properties than 3717. 3717 resembles *S. venezuelae* in giving negative Indol test and in rapid liquification of gelatin.

Utilization of Carbon Sources: Of the 15 carbon sources tested, all the five isolates failed to utilize rhamnose, lactose and xylose (Table II). Under the conditions of the experiment 3716 and 3440-14 were identical in their capacity to utilize various carbon sources except for some quantitative differences whereas 3717 was more exacting in this respect, inulin, sorbitol, mannitol, inositol, salicin, and sodium citrate not being utilized. 3717, however, readily utilized sodium succinate and in this respect resembled *S. venezuelae* and *S. virginiae*. *S. virginiae* and *S. venezuelae* seem to resemble each other in their capacity to utilize the carbon sources but they differed from 3717 in more readily utilizing starch.

Utilization of Nitrogen Sources: There was no significant difference in the capacities of the five cultures to utilize the various nitrogen sources except that 3717 utilized sodium nitrate and ammonium sulphate much better than the rest (Table III).

DISCUSSION The comparative studies of five representative strains of the *Streptomyces lavendulae* group have shown that they are closely related in various morphological, cultural and physiological properties. Certain differences in their utilization of various carbon sources were, how-

ever, observed, the isolate 3717 being more exacting in its nutritional requirements. The present studies seem to support the view that all the five isolates be included as strains of the species *S. lavendulae*. But in recent times the antibiotic producing capacity of Streptomycetes has been considered as one of the chief bases for speciation of the genus (Duggar *et al* 1954, Hasseltine *et al* 1954, Jones 1954). In this respect the creation of the species *S. venezuelae* and *S. virginiae* may be justifiable as the antibiotics produced by the two species are much different in their biological and chemical properties as compared to the streptothricin group of antibiotics produced by *S. lavendulae*. Strains of *S. roseochromogenous*, (Krainsky *emend.* Jensen) Waksman and Henrici which are reported to be closely related to *S. lavendulae* in their morphological and cultural characters, produce only streptothricin-like substances (Kurosowa, 1951) Hence this can be included under the species, *S. lavendulae*. Also, isolates 3716 and 3717, produce mycothricin complexes A and B, respectively the substances being related to streptothricin in their properties. Isolate 3716 resembles *S. lavendulae* No. 3440–14 in most respects and so is included as a strain of *S. lavendulae*. But 3717, though resembling the other four isolates in several respects, differs from them in being more exacting in its carbon utilization. Since this difference alone is not sufficient to classify it under a new species it is also included as a strain of *S. lavendulae*.

Summary

Comparative studies on five antibiotic producing isolates of the *Streptomyces lavendulae* group, viz., *S. lavendulae* 3440–14 producing streptothricin, *S. venezuelae* producing chloromycetin, *S.virginiae* producing actithiazic acid, isolates 3716 and 3717 producing mycothricin complexes A and B, respectively, have revealed that they are closely related in their morphological, cultural and physiological properties thus justifying their inclusion under the broad group of *S. lavendulae*. On the basis of the present studies the isolates 3716 and 3717 producing mycothricin complexes A and B, respectively, are classified as strains of the species *S lavendulae*. The creation of the two new species, *S. venezuelae* and *S. virginiae*, under the group can be justified only on the basis of their distinct antibiotic producing capacities.

Acknowledgement. The author is thankful to Dr. Selman A. Waksman, N. L., Professor Emeritus Institute of Microbiology, New Brunswick,New Jersey, U. S. A. for providing the facilities and for his critical review of the work.

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TABLE I. A comparison of the cultural characters of *S. lavendulae* group on various media

	Growth and Reaction				
	Isolate No. 3716	Isolate No. 3717	*S. lavendulae* No. 3440–14	*S. venezuelae** No. 3534	*S. virginiae*** No. 3651
Gelatin	liquified in 10 days; dark brown pigment produced	rapid liquification within 3 days; dark brown to black pigment produced	liquification in 10 days; dark brown pigment produced	rapid liquification; dark brown pigment produced	slow liquification; greyish brown pigment produced
Litmus milk	Submerged growth; alkaline reaction; peptonization; brown pigment produced	surface growth; strong alkaline reaction in 2 days; dark brown pigment produced	cream coloured surface growth; peptonization; alkaline reaction; brown pigment produced	alkaline reaction; peptonization; dark brown pigment produced	alkaline reaction; no coagulation; dark brown pigment produced
Nitrate broth	reduced in 3 days	reduction after 10 days	reduction in 3 days	reduction	reduction
Tryptone broth	submerged growth; light brown pigment produced	cream coloured surface growth; dark brown pigment produced	submerged growth; light brown pigment produced	black pigment produced	...
Indol test	positive in 5 days	negative even after 15 days	positive in 5 days	negative	...
Starch agar	rapid hydrolysis	week hydrolysis	rapid hydrolysis	hydrolysis	weak hydrolysis
Potato plug	black pigment	grey pigment	black pigment	dark pigment	greyish brown pigment
Glucose asparagine agar	white growth with yellowish brown pigment	greyish white growth; no pigment	greyish growth; no pigment; lavender spores	sparse growth	cream coloured growth; no pigment; no sporulation

Czapeck's agar	yellow to cream coloured growth; no pigment; lavender spores	hyaline growth; no pigment; pink spore	colourless growth; no pigment; lavender spores	...	sparse growth; no pigment; white to lavender spores
Calcium malate agar	shining deep blue pinheads; white to lavender spores	yellowish brown growth; no pigment; white spores	dull yellow to cream coloured growth no pigment; no sporulation	...	cream coloured growth; no pigment; greyish to lavender spores
Bennett's agar	sparse colourless growth; no pigment; white to lavender spores	thin colourless growth; yellow soluble pigment; abundant white spores	yellow growth; no pigment; lavender spores	...	...
Yeast extract glucose agar	dark brown pigment with deep blue pinheads; white to lavender spores	dark brown pigment; lavender to pink spores	dark brown pigment; white to lavender spores	...	...
Nutrient agar	cream coloured growth light brown pigment; no sporulation	greyish growth with white center; dark brown pigment	cream coloured growth; dark brown pigment; no sporulation	...	...
Tyrosine agar	shining dark yellow growth: light brown pigment; no sporulation; tyrosine utilized	white growth; dark blue to black pigment; abundant sporulation; tyrosine not utilized	cream coloured growth; light brown; pigment no sporulation; tyrosine utilized	...	...

*from Ehrlich *et al* (1948)

**from Grundy *et al* (1952)

TABLE II. Utilization of carbon sources by *S. lavendulae* group (Growth* after 10 days: dry weight of mycelium mg/100 ml)

C-source	Isolate No. 3716	Isolate No. 3717	*S. lavenduiae* No. 3440–14	*S. venezuelae* No. 3534	*S. virginiae* No. 3651
D-glucose	105	136	29	83	53
L-rhamnose	–	–	–	–	–
D-maltose	130	43	131	148	195
D-lactose	–	–	–	–	–
D-cellobiose	33	49	94	182	58
D-xylose	–	–	–	–	–
D-raffinose	70	65	40	60	51
Inulin	79	–	43	108	182
D-sorbitol	59	–	24	–	–
D-mannitol	69	–	42	46	93
Inositol	26	–	30	60	–
Salicin	123	–	27	71	45
Na-citrate	–	–	54	–	–
Na-succinate	–	103	–	91	61
Starch	90	43	103	143	116

TABLE III. Utilization of nitrogen sources by *S. lavendulae* group (Growth* after 10 days: dry weight of mycelium, mg/100 ml)

N-source	Isolate No. 3716	Isolate No. 3717	*S.lavendulae* No. 3440-14	*S.venezuelae* No. 3534	*S. virginiae* No. 3651
L-glutamic acid	229	253	134	140	161
DL-aspartic acid	100	177	57	105	74
DL-asparagine	122	185	91	132	77
Amino acetic acid	119	147	79	123	105
DL-tryptophane	30	39	41	38	36
L-histidine	155	199	37	102	89
L-arginine	120	212	134	134	82
DL-tyrosine	–	–	–	–	–
B-alanine	43	174	76	141	27
Urea	75	63	72	62	57
Na-nitrate	45	139	26	46	33
Am-sulphate	55	164	20	47	35

*Pridham and Gottlieb's basal medium; grown under submerged aerated conditions on shake machine at 28°C.

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AN UNDESCRIBED SPECIES OF SPHAERULINA ON IVY

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(Accepted for publication November 15, 1958)

Among some of the recent collections of plant diseases made at Simla we came across an ascomycetous fungus parasitising Ivy (*Hedera nepalensis* K. Koch). The fungus forms localised necrotic spots which are tan-coloured, with dark brown raised margins, later turning ashy and getting studded with black dot-like perithecia. The fructifications are found only on the upper surface of the leaf. The spots are angular in outline, delimited by veins and measure 4 - 13 x 4 - 8 mm. The perithecia are numerous, scattered singly, innate-erumpent, dark-brown, globose and measure 73 - 122 x 70 - 105 μ (mostly 87.5 x 77 μ). The perithecia have well-developed, broad ostiolar disc, protruding outside the epidermis measuring 52.5 to 88.0 μ in diameter. The perithecial wall is many layered thick, the outer cells of which are dark brown, parenchymatous and sterile; while the inner cells are hyaline and fertile and bear asci only,without any paraphyses.(Fig. 1.) When a perithecium is crushed, all its asci come out in a bunch. The asci (Fig. 2-a) are hyaline, cylindric-fusiform, shortly stalked, tapering below and measure 45 - 87 x 7.5 - 12 μ (mostly 45 -52 x 7.5 μ). The ascospores (Fig. 2-b) are found in a group and are eight in number in each ascus. These are hyaline, single celled, straight or somewhat curved with slightly tapering blunt ends and measure 30-36 μ in length and 3 μ in breadth. Thus the ratio between the length and breadth of the ascospores is 10 - 12: 1. The ascospores have many vacuoles which sometimes give the appearance of false septa.

Development of the Perithecium. Sections through the diseased spots at various stages of development show that the mycelium is intercellular and septate and collects just beneath the epidermal cells, gradually forming a spherical mass in the cavity within the palisade layer of the host tissue. The adjoining cells turn dark brown and gradually collapse giving rise to necrotic spots. The fungus then forms a dark sclerotioid mass which gradually develops a central cavity, the inner layers of which on further development bear asci and ascospores, while the outer layers at the top portion grow and pierce through the epidermis. The apical cells become opaque black, grow massive to form a disc which surrounds a narow ostiole. The central portion of the disc grows upwards and forms a papilla which gives the disc a lobed appearance The perithecia are formed singly. The body of the perithecium remains immersed in the host tissue below the epidermis in the region of the palisade cells. The asci differentiate themselves into cylindric fusiform structures arising from a common point in the basal region of the perithecial cavity, the central ones are borne directly on the perithecial wall, while the lateral ones develop short stalks. At no stage the presence of paraphyses or paraphysoids was observed. The asci have a single hyaline wall and when young, have diffuse protoplasmic contents, which in due course are delimited into ascospores. The ascospores are single celled, straight

or somewhat curved, and are grouped together in the ascus like a bundle of faggots. They resemble somewhat the broad type of *Septoria* spores but have blunt ends.

TAXONOMY OF THE FUNGUS. The development of the perithecium is truely that of the Mycosphaerellaceae of Sphaeriales, though the opening of the ostiole at times appears similar to that of Hysteriales, possibly due to the tough epidermis of the host. When the perithecia are crushed, the asci come out in a bunch. The ascospores though aseptate, are typical of Hyalophragmiae regarding their size and shape and would thus warrant the inclusion of this fungus in *Sphaerulina* Sacc. No species of this genus has been described so far on *Hedera nepalensis*. It is, therefore, proposed to name this fungus as *S. hederae*.

Sphaerulina hederae sp. nov. Maculae alutaceae, marginibus brunneis elevatisque, nervis definitae, ambitu angulares, distinctae perithecis nigris puncti similibus in superiore pagina foliorum. Perithecia plurima, dispersa, innato-erumpentia, efformata, singula ut plurimum in textibus vallaribus, globosa, fusce brunnea, magnitudinis 73 - 122 x 70 - 105 μ (ut plurimum 87.5 x 77 μ) ostiolata; ostiolum compressum, magnae mollis, lobatum, se protrudens, magnitudinis 52.5 - 88 μ diam. Parietes perithecii pluribus seriebus crassi; cellulae exteriores fusce brunneae, parenchymaticae atque steriles; cellulae interiores hyalinae, fertiles, producentes ascos. Asci hyalini, cylindric-fusiform, brevi-pedicellati, fastigati infra, octospori, paraphysibus nullis, magnitudinis 45 - 87 - x 7.5 - 12 μ(ut plurimum 45 - 52 x 7.5 μ). Ascosporae simul aggregatae in asco more sarmentorum in fascibus, hyalinae, unicellulatae, rectae vel aliquantum curvatae, tenuiter fastigatae in utrumque apicem, apicibus hebetibus, 30-36 μ longae, 3 μ latae, ratione longitudinem inter et latitudinem 10-12: 1, vacuolatae; vacuolis plurimis atque septa falsa simulantibus.

Typus lectus in foliis *Hederae nepalensis*, e familia Araliacearum, in "Catchment Area", in loco Simla, in provincia Punjab, die 3 mensis augusti, anii 1955, a C. L. Sethi, et positus in Herbario Crypt. Indiae Orientalis, I. A. R. I., in urbe New Delhi, sub numero 23750, et Herb., C. M. I., Kew, England (Sub numero 61311).

Our grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi for encouragement and keen interest throughout this work. We are also indebted to Mr. E. W. Mason, Mycologist, Commonwealth Mycological Institute Kew, for helpful suggestion in the identification of this fungus and to Dr. H. Santapau for the latin diagnosis.

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Fig. 1

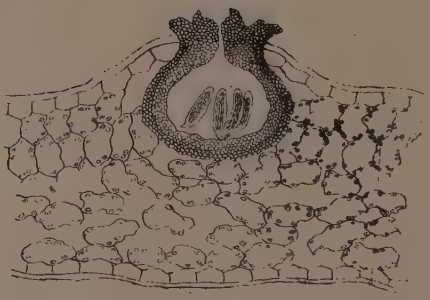

Fig. 2

Fig. 1. *Spaerulina hederae.* A section through a perithecium (semidiagrammatic) showing asci and ascospores.

Fig. 2. *Sphaerulina hederae.* (a) Ascus. (b). Ascospores x 1800.

EFFICACY OF DIFFERENT FUNGICIDES 1. SEED DISINFECTION IN RELATION TO STEM ROT OF JUTE AND STRIPE DISEASE OF BARLEY

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(Accepted for publication November 10, 1958)

One of the major factors contributing to the initiation of primary infection of plant diseases is seed borne-infection. Sometimes a heavy spore load is carried over the seed externally from the heavily infected field to certain areas where the disease is not known, resulting in severe primary infection of the crop. In stem rot of the jute (*Macrophomina phaseoli* (Maubl.) Ashby) seed borne-infection is more important in the initiation of the disease as has been shown by Varda Rajan and Patel (1946). Similarly in stripe disease of barley (*Helminthosporium gramineum* Rabenh.), the disease spreads primarily through infected seed. Control of such infection has been achieved by the fungicidal seed dressings in certain cases. A large number of proprietary as well as other fungicides are pouring into the market in our country and in order to find out the most effective seed dressing fungicide for the control of the above two diseases, various organic mercurial and non-mercurial fungicides were tried.

It is not possible to test the comparative efficacy of large number of fungicides under field conditions because the process requires a fairly long period, good amount of labour and is dependent upon vagaries of nature before any definite conclusions can be drawn. Therefore, for testing the comparative efficacy of different fungicides a simple and rapid method which needs only laboratory equipment was followed. The technique used is a modification of Ulster method which has been successfully followed by Muskett and Colhoun (1943) and has been reported to predict to a great extent the field performances of fungicides. Using this technique of screening fungicides some of the ineffective products could be eliminated from field trials.

Seeds of jute (variety JRC 412) severely infected with *Macrophomina phaseoli* obtained from the Central Jute Research Station, Barrackpore, on testing showed 100 per cent infection. Attempts were made to get the naturally infected barley seed but it was found that the disease was not uniformly present on all the seeds and the seed infection ranged only from 10-20 per cent. The use of such a seed was not considered suitable for obtaining reliable data. Seeds (variety NP 3) artificially infected with spores of *Helminthosporium gramineum* were, therefore, used for the purpose. Nineteen lots of jute and barley seeds were treated with various fungicides at the doses given in Tables I & II. The seed and the fungicides were mixed in flasks by shaking them on an electric shaker for 15 minutes and were then plated on potato dextrose agar* and the petri plates were incubated at 25 °C. The seeds showing fungus growth around them on the

*Peeled potato 200 gms; Dextrose 20 gms; Agar agar 20 gms; Distilled water 1000 c.c.

TABLE. I. Showing comparative efficacy of various fungicides against seed borne infection of stem rot of jute (*Macrophomina phaseoli* (Maubl.) Ashby) at three different doses.

S. No.	Fungicide	Active ingredient	I Experiment			II Experiment			III Experiment		
				% of seeds			% of seeds			% of seeds	
			Dose	with fungus growth	germination	Dose	with fungus growth	germination	Dose	with fungus growth	germination
1.	N.I. Ceresan	ethylmercury phosphate	0.25%	25	78	0.40%	5	85	0.60%	0	82
2.	Arasan	tetramethylthiuram disulphide	0.25%	95	84	0.45%	28	80	0.60%	18	87
3.	2% Ceresan	ethylmercury chloride	0.35%	50	86	0.50%	36	85	0.75%	11	87
4.	Semesan	2-chloro-4-(hydroxymercuri) phenol	0.375%	50	82	0.50%	41	77	0.75%	22	80
5.	Hexasan	organically combined mercury	0.35%	50	86	0.50%	49	80	0.75%	22	80
6.	Cerenox	organic non-metallic compd.	0.50%	100	86	0.75%	100	87	1.0 %	100	88
7.	Agrosan GN	tolylmercury acetate	0.35%	57	85	0.50%	41	79	0.75%	9	83
8.	Harvesan	organically combined mercury	0.30%	37	85	0.50%	27	85	0.75%	19	84
9.	Lunasan	1-(ethylmercuri)-2-thiourea	0.35%	100	85	0.50%	100	78	0.75%	100	81
10.	Fusariol	ethylmercury cyanide	0.25%	30	88	0.40%	31	87	0.60%	4	82
11.	Flit 406	n-(trichloro methylmercapto)-4-cyclohexene-1,2-dicarboximide	0.25%	25	90	0.40%	6	85	0.50%	0	78
12.	Tillex	ethylmercury chloride	0.25%	100	73	0.40%	100	83	0.60%	27	84
13.	Spergon	tetrachloro-p-benzoquinone	0.50%	100	85	0.75%	47	81	1.00%	31	76
14.	Thiram	tetramethylthiuram disulphide	0.375%	37	85	0.50%	28	86	0.75%	17	83
15.	Phygon	2-3-dichloro-1, 4-napthoquinone	0.25%	40	82	0.40%	38	80	0.60%	19	89
16.	Dow 9B	zinc trichlorophenate	0.30%	45	82	0.50%	42	82	0.75%	40	92
17.	Agrosan 5W	organically combined mercury	0.30%	10	78	0.40%	3	80	0.60	0	88
18.	Fernasan A	tetramethylthiuram disulphide	0.50%	40	80	0.75%	30	92	0.85%	13	75
19.	Leytosol B	phenylmercuryurea	0.25%	45	88	0.40%	32	93	0.60%	18	82
20.	Control	(untreated)	...	100	78	...	100	79	...	100	82

TABLE: II. Showing comparative efficacy of different fungicides for the control of stripe disease of barley (*Helminthosporium gramineum* Rabenh.)

S. No.	Fungicide	Active ingredient	dose	Percentage of seeds showing fungus growth			Percentage germination of seeds
				Expt. I	Expt. II	Expt. III	
1.	Arasan	tetramethylthiuram disulphide	0.30%	6	8	10	80
2.	N. I. Ceresan	ethylmercury phosphate	0.07%	50	52	54	100
3.	2%Ceresan	ethylmercury chloride	0.18%	6	4	6	100
4.	Cerenox	organic non-metallic compound.	0.22%	100	98	100	56
5.	Harvesan	organically combined mercury	0.30%	6	6	8	84
6.	Spergon	tetrachloro-p-benzoquinone	0.22%	48	66	72	78
7.	Tillex	ethylmercury chloride	0.18%	82	92	100	100
8.	Thiram	tetramethylthiuram disulphide	0.30%	14	12	16	100
9.	Lunasan	1–(ethylmercuri)–2–thiourea	0.30%	100	96	100	70
10.	Phygon	2–3 dichloro–1, 4–napthoquinone	0.15%	46	50	50	80
11.	Fusariol	ethylmercury cyanide	0.18%	0	0	0	100
12.	Dow 9 B	zinc trichlorophenate	0.18%	72	94	96	100
13.	Agrosan GN	tolylmercury acetate	0.30%	0	0	0	100
14.	Fernasan A	tetramethylthiuram disulphide	0.30%	12	10	14	100
15.	Brassicol	pentachloronitrobenzene	0.07%	86	96	94	100
16.	Flit 406	n-(trichloromethylmercapto)-4-cyclohexene-1, 2-dicarboximide	1.18%	16	30	36	100
17.	Hexasan	organically combined mercury	0.30%	94	96	100	90
18.	Leytosol B	phenylmercuryurea	0.07%	60	62	86	100
19.	Agrosan 5W	organically combined mercury	0.15%	0	0	0	50
20.	Control	no treatment	—	100	100	100	80

medium were counted at the end of six days. The absence of fungus growth around the seed was taken as an indication of the effectiveness of the fungicide. Suitable controls were kept alongwith the different treatments. The germination of the treated as well as the untreated seeds was also recorded. The results regarding the efficacy of different fungicides and their effect on germination of seeds are summarised in Table I & II.

It is evident from Table I that in case of *M. phaseoli* the doses in the range of 0.25 to 0.5 per cent of seed-weight could not completely check the growth of the fungus. In the second experiment also none of the fungicides could completely check the disease though the control of the disease was comparatively better with Agrosan 5W, N. I. Ceresan and Flit 406 where the infection was 3, 5 & 6 per cent, respectively. In third experiment doses were further increased to see if any of the fungicides could completely control the disease without having deleterious effect on the germination of the seed. The results indicate that Agrosan 5W, Flit 406 and New Improved Ceresan can completely check the growth of the fungus at higher doses without impairing the germination of seed. Fusariol, Agrosan GN and 2% Ceresan were next in order of merit giving fairly good control of the disease.

The results in case of *H. gramineum* (Table II) indicate that Agrosan GN, Agrosan 5W and Fusariol completely checked the growth of the fungus. In seeds treated with Arasan, 2% Ceresan, Harvesan, Ferrasan and Thiram the infection ranged from 6 to 16 per cent. In most cases germination of seeds was enhanced as compared to that of control while in Agrosan 5W and Cerenox it was markedly suppressed.

Summary

Tests were conducted in the laboratory to study the comparative efficacy of nineteen seed dressing fungicides for the control of seed borne infection of stem rot of jute (*Macrophomina phaseoli*) and stripe disease of barley (*Helminthosporium gramineum*). It was observed that New Improved Ceresan, Flit 406 and Agrosan 5W were able to control stem rot of jute whereas Fusariol, Agrosan GN and Agrosan 5W were found effective against stripe disease of barley.

Acknowledgments. Our grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology for suggesting the problem, keen interest and valuable guidance during the course of this work.

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THE CORRELATION BETWEEN THE LEAF AND NECK INFECTIONS OF THE BLAST DISEASE OF RICE

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(Accepted for publication November 20, 1958)

Introduction. *Piricularia oryzae* Cav., the causal organism of the blast disease of rice, is known to infect the crop at various stages of its growth. It causes seed-rot, seedling blight, leaf-spot, node infection, neck infection, and grain infection. The disease spreads in stages starting either from the infected seed, the diseased plant debris present in the field or through the alternative hosts, depending upon the prevalence of the favourable climatic conditions. It is generally believed that the neck infection of the disease runs parallel to the leaf infection (Krishnaswami *et al* 1951). It is quite probable that when the leaves are severely infected, the emerging panicle is subject to heavy attack by the fungus. But a variety may react differently to the leaf and neck infections due to its inherent characters. According to Anderson *et al* (1947) even a susceptible variety may become resistant to the disease in the late tillering and early heading stages.

With a view to examine whether there is any correlation between the leaf and neck infections of the disease, the data on ten varieties of rice collected over a period of ten years were studied. The question, whether the leaf infection leads to the neck infection of *P. oryzae*, was also examined with the data and the results are presented.

Material and Methods. Ten varieties of rice, with varying degrees of susceptibility to the blast disease, were selected for this purpose. The details regarding the method of cultivation of the crop, lay out of the experimental plots, manurial and other treatments and the collection of the data on disease incidence are the same as described by Krishnaswami *et al* (1951). The varieties were grown in the O Block, Central Farm Wetlands, Agricultural Research Institute, Coimbatore, during the main crop season (August to February) every year from 1943-44 to 1952-53. Each treatment (variety) consisted of 21 plants in a single row, with a spacing of one foot between plants, and replicated four times by the Square Lattice design.

The leaf infection of the blast disease was graded as nil, low, medium, and heavy, according to the standard chart as described by Krishnaswami *et al* (1951) and category values of 0, 2, 5, and 10 respectively, were assigned. The leaf infection percentage in each case was calculated by taking the category value of 210, the maximum possible value that could be obtained when all the 21 plants in a treatment are heavily infected, as 100 per cent infection. These observations were recorded at monthly intervals and the

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final counts, made prior to the earhead emergence, were taken for the purpose of the present studies. The neck infections were recorded at the time of the harvest and the percentage infection in each treatment obtained by counting the total number of the diseased and healthy tillers from all the 21 plants in a treatment.

The experimental data were statistically analysed by the Variable Square method (Patterson 1939) for any correlation between the leaf and neck infections. The data on each variety were analysed separately as also the overall correlation for the average figures of all the ten varieties. For the analysis on individual varieties, 40 observations, comprising four replications every year for the ten year, and for the overall correlation 100 observations, comprising the average figures for the ten varieties for the ten years, were analysed.

Results. The percentages of leaf and neck infections for the ten varieities of rice for the ten year period, 1943-44 to 1952-53, are presented in Tables I and II. These figures represent the average of the four replications under each treatment. During the three year period, 1947-50, there was no appreciable disease incidence even in the highly susceptible varieties, which is probably due to the unfavourable climatic conditions.

A comparison of the results in the two Tables shows that the heaviest leaf infection occurred during 1951-52 and the heaviest neck infection during 1952-53. On the basis of their susceptibility to the leaf and neck infections, the ten varieties can be arranged in the descending order as follows:

Leaf Infection : Adt. 10, Co. 13, Co. 7, Bam. 1, Co. 18, GEB. 24, Bam. 2, Adt. 21, Adt. 22, Ptb. 4.

Neck Infection : Adt. 10, Co. 13, Bam. 1, GEB. 24, Bam. 2, Co. 7, Co. 18, Adt. 22, Adt. 21, Ptb. 4.

The varieties Adt, 10 and Co. 13 are highly susceptible to both the types of infections, Co. 7, Co. 18, GEB. 24, Bam. 2, and Bam. 1 moderately susceptible, and Adt. 21, Adt. 22, and Ptb. 4 relatively less susceptible to the infections.

The results of the statistical analysis of the data for the correlation between the leaf and neck infections are summarised in Table III.

There is highly significant positive correlation between the leaf and neck infections when all the ten varieties are considered as a lot. Among the individual varieties, there is positive correlation between the two types of infections in the more susceptible varieties, viz., Adt. 10, Co. 13, Co. 18, Co. 7, and GEB. 24, whereas in the less susceptible varieties there is no positive correlation. But this does not necessarily indicate that whenever there is high leaf infection it is associated with heavy neck infection and *vice versa* (Fig. 1). In the variety Adt. 10, the neck infection was considerably low during 1943-44 inspite of 100 per cent leaf infection during that year (Fig. 2). In GEB. 24 there was high neck infection during 1952-53 though the leaf infection percentage was considerably low (Fig. 2).

TABLE I. The average percentage of leaf infection of *P. oryzae* in ten varieties of rice during 1943–53

Variety	1943–44	44–45	45–46	46–47	47–48	48–49	49–50	50–51	51–52	52–53	Total	Average
Adt. 10	100	70	33	27	0	0	11	91	100	49	481	48.1
Adt. 21	0	3	0	1	0	0	0	5	9	0	18	1.8
Adt. 22	0	0	0	1	0	0	5	4	5	2	17	1.7
Bam. 1	0	1	2	3	0	0	0	25	23	21	75	7.5
Bam. 2	0	0	0	1	0	0	0	10	7	10	28	2.8
Co. 7	39	10	2	2	0	0	0	21	18	10	102	10.2
Co. 13	0	14	0	8	0	0	0	40	80	15	157	15.7
Co. 18	8	1	0	1	0	0	0	14	10	12	46	4.6
GEB. 24	0	1	1	2	0	0	0	11	13	6	34	3.4
Ptb. 4	0	0	0	1	0	0	0	0	1	1	3	0.3

TABLE II. The average percentage of neck infection of *P. oryzae* in ten varieties of rice during 1943–53

Variety	1943–44	44–45	45–46	46–47	47–48	48–49	49–50	50–51	51–52	52–53	Total	Average
Adt. 10	24	39	22	32	9	0	8	100	53	65	352	35.2
Adt. 21	12	12	2	2	0	0	1	2	1	11	43	4.3
Adt. 22	5	18	3	1	0	0	1	2	0	15	45	4.5
Bam. 1	23	26	9	10	0	0	0	7	1	15	91	9.1
Bam. 2	11	18	4	4	0	0	1	10	3	25	76	7.6
Co. 7	21	15	3	3	0	0	0	7	3	18	70	7.0
Co. 13	20	25	16	20	0	0	4	53	100	51	289	28.9
Co. 18	21	14	4	5	0	0	1	7	5	12	69	6.9
GEB. 24	10	12	3	3	0	0	0	15	7	28	78	7.8
Ptb. 4	9	11	4	2	0	0	0	0	1	11	38	3.8

TABLE III. Coefficients of correlation between the leaf and neck infections of *P. oryzae* in ten varieties of rice

Variety	Number of samples (n)	Correlation Coefficient (r)	r–values from tables		Significant or not
			5% level	1% level	
Adt. 10	40	0.6369	0.304	0.393	**
Adt. 21	40	0.0498	0.304	0.393	no
Adt. 22	40	0.1171	0.304	0.393	no
Bam. 1	40	0.0089	0.304	0.393	no
Bam. 2	40	0.3164	0.304	0.393	*
Co. 7	40	0.4194	0.304	0.393	**
Co. 13	40	0.8241	0.304	0.393	**
Co. 18	40	0.6657	0.304	0.393	**
GEB. 24	40	0.3775	0.304	0.393	*
Ptb. 4	40	0.2432	0.304	0.393	no
Average value for the ten varieties	100	0.7044	0.195	0.254	**

**Significant at 1% level
*Significant at 5% level

DISCUSSION. The results obtained in the present studies indicate that there is a positive correlation between the leaf and neck infections of *P. oryzae* on rice. This seems to be especially true in the cases of the more susceptible varieties, Adt. 10, Co. 13, Co. 7, and Co. 18. These results also seem to support the views of Krishnaswami *et al* (1951) that the leaf and neck infections run parallel to each other. It is clear from the results that the varieties are almost equally susceptible to the two types of infections, when tested individually over a period of years. In the less susceptible varieties, however, there is no positive correlation between the leaf and neck infections.

Anderson (1947) tested rice plants for susceptibility to *P. oryzae* at various stages of growth and found that some varieties are most susceptible to the infection in the seedling, early tillering and heading stages. They also found that some susceptible varieties became resistant to the disease in the late tillering and early heading stages. In the present studies it has been observed that the heavy leaf infection in a variety was not always followed by heavy neck infections. Considering that the interval between the times of recording of the final leaf infection and the neck infection is over a month, it is possible that some external factors like relative humidity and atmospheric temperature influence the development of the symtoms of infection. These factors may also be responsible for the absence of the leaf infections in the early stages and the subsequent incidence of neck infections. It may also be true that some internal factors like morphological and physiological properties of the plants play a role in the development of the resistance in the late stages of the crop (Abe 1937, Anderson *et al* 1947). This might partly be the reason for the variations in the reactions to the two types of infections in the less susceptible varieties under the present studies.

SUMMMARY

The data on the incidence of the leaf and neck infections of the blast disease of rice caused by *Piricularia oryzae* Cav. on ten varieties of rice with varying degrees of susceptibility to the disease, collected over a period of ten years, from 1943-44 to 1952–53, were analysed for any correlation between the two types of infections. There was highly significant positive correlation between the leaf and neck infections, when the average values for all the ten varieties were analysed. Among the individual varieties, there was positive correlation only in more susceptible varieties, Adt. 10, Co. 13, Co. 7, Co. 18, Bam. 2, and GEB. 24, the less susceptible ones showing no significant correlation.

A given variety seems to be equally susceptible to both leaf and neck infections of *P. oryzae*, when tested over a period of ten years. In a given year, however, the heavy incidence of leaf infection was not always associated with a proportionately high neck infection and *vice versa*.

ACKNOWLEDGMENT: The data on the incidence of the disease for the period, 1949–53, were collected by the authors and the rest by the other workers under the scheme for the investigation of blast disease of rice in Mad-

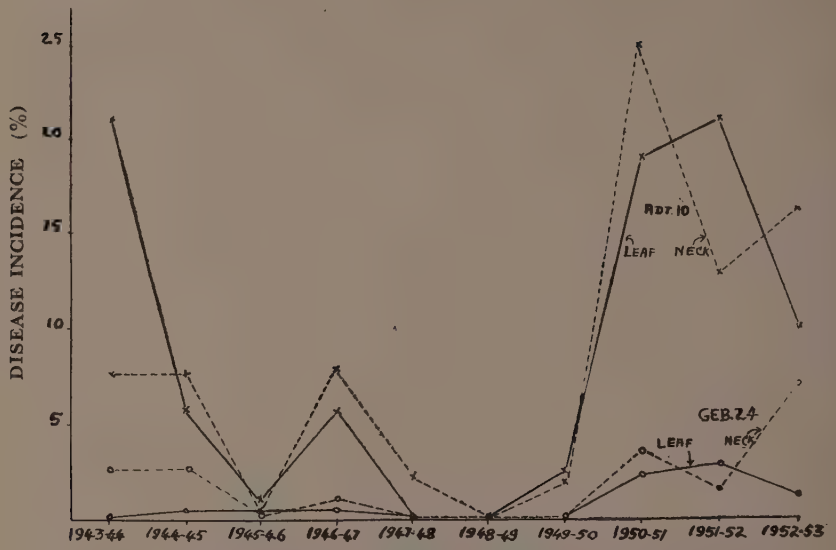

Fig. 1. The correlation between the average incidence of leaf and neck infection of *Piricularia oryzae* in ten susceptible varieties oi rice.

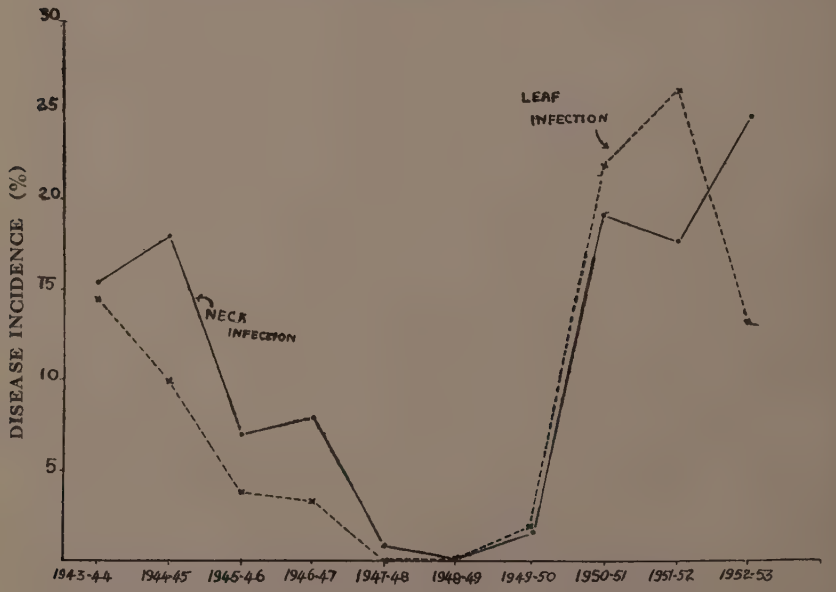

Fig. 2. A comparison of the incidence of leaf and neck infections of *P. oryzae* in Adt. 10 and GEB 24.

ras State, which was partly financed by the Indian Council of Agricultural Research. The authors are thankful to Sri C. M. Bakthavathsalu for his help in the statistical analysis of the data and to Dr. K. Ramakrishnan for his encouragement in these studies.

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Phytopathological Notes

A Convenient method of Storage of Fungal Cultures. by R. K. Singh. During the course of investigations, it was found that cultures of *Fusarium moniliforme* Sheldon, *Fusaruim caruleum* (Lib.) Sacc. and *Fusarium avenaceum* (Fr) Sacc. could be safely and conveniently preserved for longer periods in soil medium consisting of sieved soil and glucose. 1 per cent glucose was added to the sieved soil and thoroughly mixed. 10 gms. of the soil was taken in each culture tube and made into slants by carefully tapping them. Water was added to the culture tubes carefully just to moisten the whole soil surface. The culture tubes were then plugged with cotton and sterilised at 15 lbs. pressure for 20 minutes in the autoclave.

Culture tubes were inoculated with the fungus and allowed to grow for a week at optimum temperatures (25–28 °C.). The culture tubes were then stored at -18 °C. It was observed that the cultures remained viable for over 2 years under these conditions without undergoing any change.

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Cercospora Leaf Spot of Sesame (*Sesamum orientale* **L.**) by N. N. Mohanty. Sesame is grown as a major oilseed crop in Orissa having an acreage of 2,54,800 acres out of the total area of 5,63,800 acres under oilseeds. It forms the chief source of a sweet oil extensively used for domestic purposes.

During August, 1957, a severe leaf spot disease of sesame was noticed at the State Agricultural Research Station, Bhubaneswar. In the early stages, the disease manifests itself in form of minute chlorotic lesions on the upper surface of the leaves and later, when the affected tissues become necrotic, the colour of the spots changes to dark brown, whereas on the corresponding lower surface of the leaves, the colour of the spots remains olivaceous brown. The spots increase in size and, delimited by veinlets of the leaves, become angular. In case of severe infection, several spots develop on the leaf blade, as a result of which, such leaves dry up and shed causing defoliation of the plant. The disease has been observed to occur on Kharif crop but not on Rabi crop.

The infected specimens were examined and found to be incited by a new species of *Cercospora*.

Cercospora sesamicola sp. nov.

Leaf spots angular, limited by leaf veinlets, 1 to 8 mm. in diameter, brown; fruiting on both the surfaces, but chiefly on the lower surface; stromata dark brown, subglobular, 20 to 46μ in diameter; fascicles closely packed, conidiophores olivaceous brown, simple, 0- to 2-septate, straight to slightly curved, tip sub-truncate, 12.5 to 36 x 3 to 4.5μ. Conidia hyaline, cylindric, straight to mildly curved, base truncate to sub-truncate, tip obtuse, indistinctly 2- to 7-septate, 20 to 120 x 2 to 2.8 μ (Fig. 1)

On the living leaves of *Sesamum indicum* L; State Agricultural Research Station, Bhubaneswar (Orissa), 28–8–57, N. N. Mohanty. Type deposited in the Herbarium of Mycology and Plant Pathology Section, Bhubaneswar and in Herb. Crypt. Ind. Orient, I. A. R. I., New Delhi.

Cercospora sesamicola spec. nov.

Maculae foliorum angulares, circumscriptae venulis foliorum, 1 to 8 mm. diam brunnae; fructificantes in utraque pagina sed praesertim in inferiore; stromata fusce brunnea, subglobosa, 20 to 46 μ diam; fasciculi arcte dispositi; conidiophori olivaceo - brunnei, simplices, 0 - 2 - septate, recti vel paulum curvati, apicum subtruncata 12.5 - 36 x 3- 4.5μ; conidia hyalina, cylindrica, recta vel molliter curvata, basim truncata vel sub-truncata, apicem obtusa, indistincte 2- 7-septata, 26 - 120 x 2 - 2.8 μ

In foliis viventibus *Sesami indici* Linn. in Statione agricola status ad Bhubaneswar in Statu Orissa, die 28 augusti anni 1957, N. N. Mohanty. Typus positus in herbario Mycologico atque sectione pathologica plantarum, Bhubaneswar, et in Hrb. Crypt. Ind. Orient. I. A. R. I., New Delhi.

The species under report differs from *Cercospora sesami* Zimmermann recorded earlier on *Sesamum orientale* L. in its uniformly brown angular leaf spots, the short narrow closely packed conidiophores and the narrowly cylindric conidia.

The author is greatly indebted to Dr. Charles Chupp, Professor of Plant Pathology, Cornell University, U.S.A. for his help in identifying the fungus. The author is also grateful to Rev. Father Dr. H. Santapau, Head of the Department of Biology, St. Xaviers' College, Bombay for rendering the Latin diagnosis.

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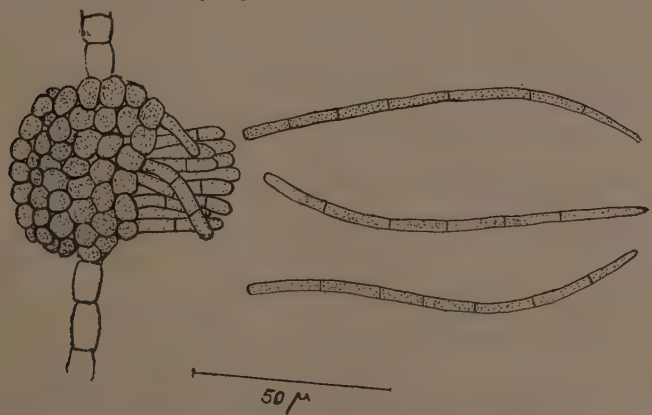

Fig. 1 Camera lucida drawing of the Stromata, Conidiophores and conidia of *Cersospora sesamicola* sp. nov.

Toxicity of the Exo-Enzyme Produced by *Fusarium moniliforme* Sheld*. by R. K. Singh. It has been pointed out by Singh & Wood (1956) that *F. moniliforme* Sheldon produces pectolytic enzymes (protopectinase) in some of the natural substrates like potato and tomato. It also produced the enzyme in synthetic media in presence of pectin or such other related compounds (Singh & Wood, 1956).

With a view to study the relationship between maceration of the vegetable tissue and its killing effect, some preliminary work was taken up. The protopectinase activity differed on the type of vegetable tissue used. The activity was tested at pH 8.0 on 0.5 mm. thick discs (or otherwise mentioned) in each case. The results are set out in Table I.

TABLE I. Effect of enzyme extract on various vegetable tissues.

Tissue.	R. T.* (mins.)	Activity 1000/R.T.
Cucumber.	5—8	125
Carrot.	25—30	33
Potato.	10—15	66
Turnip.	10—15	66
Control (no enzyme) ...	Nil	Nil

It is apparent from the above results that cucumber discs proved to be most susceptible to protopectinase and the carrot discs least. On the basis of the above results potato and cucumber discs were selected for studying the killing effect of protopectinase.

Toxicity was estimated by placing 0.5 mm. thick discs of cucumber and potato into a plasmolysing agent containing neutral red (Molar K No[3], 8.5 ml; 0.1% neutral red chloride, 1.0 ml; phosphate buffer, pH 7.6, a few drops). This was prepared freshly for each estimation as crystals were deposited from the solution at the weakly alkaline reaction which is optimal for absorption of the dye by the cells. The sections remained in this solution for 20 minutes, the plasmolysing agent preventing any further toxic action. Comparisons were made visually and the results were recorded by number: 5, whole discs covered with red spots, 4, 3, 2, 1 gradations; 0, no spots visible. The results are summarised in Table II.

*This piece of work was conducted at the Imperial College of Science Technology, London.

*R. T. (Reaction time). The time taken by the enzyme to break the coherence of the parenchymatous tissue.

TABLE II. Effect of enzyme extract on maceration and killing of plant tissues.

Tissue.	R. T. (mins)	Killing of the tissue after (mins)						
		5	10	15	20	30	45	60
Potato	10—15	5	4	4	3	2	1	0
Cucumber.	5—8	4	2	1	0	–	–	–
Control.	Nil	5	5	5	5	5	5	5

Cucumber discs were killed much more quickly than potato discs. In the first case, killing was effected after 10 minutes whereas in the latter after 5 minutes. This also suggested that the enzyme first acts on the middle lamella and macerates the tissue, and later on it kills the tissue. Similar results were obtained by Tribe (1955) and Fushtey (1953) with *Botrytis cinerea* and *Bacterium aroidae* enzyme extracts.

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Fusarium moniliforme Sheld. causing rot of vegetables* by R. K. Singh. The pathogenicity of the fungus *F. moniliforme* was tested on apples and tomato fruits, narcissus and onion bulbs, potato tubers, and carrot, turnip and swede roots.

METHOD OF INOCULATION: The inoculations were performed by the plug method, the technique of inoculation differing slightly according to type of material.

Apple, narcissus, onion, carrot, turnip and swede were surface sterilized by scrubbing with 90% alcohol which was then allowed to dry. A plug 3 mm. deep was taken out by means of a sterile cork borer (diam. 5 mm). The inoculum was placed in the hole, the plug re-inserted and the broken surface sealed with a mixture of molten wax and vaseline to check contamination and desiccation of inoculum.

* This work was carried out at the Imperial College of Science and Technology London.

Potatoes were washed thoroughly and left overnight to dry. They were surface sterilized by means of 90% alcohol and the alcohol was allowed to dry. For inoculations a plug 2mm. deep was taken out, the inoculum was placed in the hole and sealed without re-inserting the plug.

Tomatoes were surface sterilized by 90% alcohol and left to dry. A slit 4 mm. long was made by means of a sterile scalpel. The inoculum was placed in the slit which was then sealed.

Results and Conclusion: In a preliminary experiment it was found that *F. moniliforme* did not attack carrot, turnip, swede, narcissus or onion; on potato (var. Kind Edward) there was slight necrosis without measurable rotting, the lesion being cut off by a cork layer. Apples (Bramley seedling) and tomatoes were attacked, giving respectively 6.7 and 13.8 % of the tissue rotted.

The inoculation tests on apple and potato were repeated at the series of temperatures 5°, 15°, 20°, 25° and 30°C. With apples there was no rotting at 5°C.; from 15°C and upwards rotting increased reaching 5–6% of the fruit weight at 25° or 30°C. With potatoes there was no attack at 50°C; at 15–30°C it was slight, being cut off by a cork barrier.

Pretreatments for 1–2 weeks of apples and turnips at 50°C and of potatoes at 50°C and 35°C after the manner described by various workers (Vasudeva, 1930; Chona, 1932) had no noticeable effect on the amount of infection produced.

Similar negative results were obtained when the fungal inoculum was re-inforced by 1%glucose and 1%pectin.

Increase in the water content of potato cylinders likewise did not lead to progressive attack.

A comparison of the pathogenicity of *F. moniliforme*. *F. avenaceum* and *F. caeruleum* on potato tubers is set out in Table I. Old tubers (Majestic) and young (Jersey) were used for this test.

Table I. Production of Rot on old and new Potatoes.

Fungus	Type	Result of inoculation		Mean % rotted tissue.
		No. inoculated	No. infected	
F moniliforme	Old	5	5	Negligible
,,	New	,,	,,	,,
F. avenaceum	Old	,,	,,	6.3
,,	New	,,	,,	20.5
F. caeruleum	Old	,,	,,	Negligible
,,	New	,,	,,	,,

Though initial attack was shown by all the three isolates, the only one which gave a measurable amount of rotting was *F. avenaceum*.

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A New Species of Peronospora on Euphorbia Hypercifolia L. by S. B. Mathur & S. Sinha. Seven species of *Peronospora* are so far recorded in literature (Saccardo 1888, 1926; Gäumann 1923; Sawada 1927) to occur on various species of *Euphorbia*. Gäumann (1923) suspected a doubtful form of *Peronospora* on *E. hypercifolia* L. on which a new species of *Peronospora* is reported herewith. The host is an erect annual fairly abundant in Agra during the monsoon months of July, August and September and also extending to late October in this area. The parasite causes characteristic symptoms of downy mildew, forming cottony masses of conidiophores on the under surface of the leaves. Antheridia, oogonia and oospores are abundantly found in the infected leaves. Following the concept of species in obligate parasites, this new record is named *Peronospora hypercifoliae* Sinha & Mathur, sp. nov., a diagnosis of which appears below.

The specimens of the species reported here are deposited in the Herbarium of the Botany Department, Agra College, Agra and duplicates at the Herb. Crypt. Ind. Orient. of Indian Agricultural Research Institute, New Delhi.

Peronospora Hypercifoliae Sinha & Mathur, sp. nov.

Diligenter parasiticus, mycelium intercellulum, haustoria ramicula; conidiophoris gracile emergentes per stomata fasiculis, ramiculi dichotome acute angulis; conidiis ferentis singulatum in sunnis ramorum, dichotomis 5–7; conidiis globosis, viola, 16.5–19.8 16.5–19.8 μ; germinantia tubis germinum formatis; Oosporis 18.1–29.7 16.5–29.7 μ, fuscus, subglobosis, murus inaequabile densatus.

In foliis *Euphorbia hypercifolia* L., Agra, 5. 8. 1956; leg S. Sinha and S. B. Mathur, typus.

Strictly parasitic; mycelium intercellular with branched haustoria; conidiophores slender, emerging through the stomata in clusters, branching dichotomously at acute angles bearing conidia singly at tips of the ultimate branches, dichotomies 5–7; conidia globose, violet, 16.5–19.8=16.5–19.8μ, germinating by germ tubes; oospore 18.1–29.7=16.5–29.7μ, brown, subglobose with thickened wall, thickenings not uniform.

On leaves of *Euphorbia hypercifolia* L. Agra, 5. 8. 1956; leg S. Sinha & S. B. Mathur, type.

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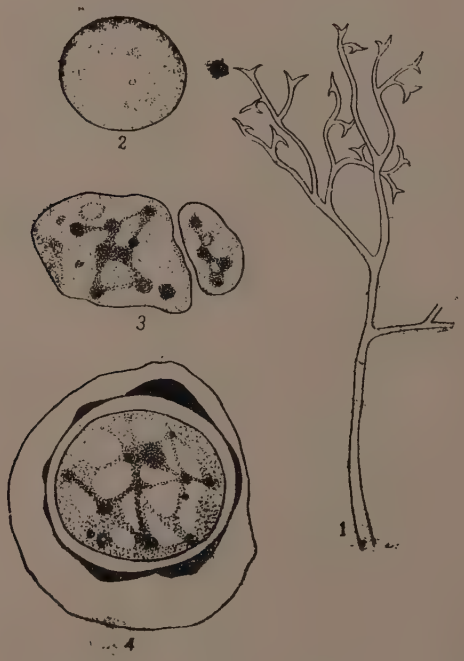

Fig. 1. Conidiophore showing dichotomous branching. X 380.
Fig. 2. Conidium. X 925.
Fig. 3. Oogonium with antheridium. X 925.
Fig. 4. Oospore within the oogonial wall. X 925.

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Two Additional Vectors of Chilli Mosaic Virus by T. K. Nariani and K. S. M. Sastry. Chilli mosaic virus was first reported in India by McRae (1924) and Kulkarni (1924). Jha and Raychaudhuri (1956) studied the host range and physical properties of the causal virus at Delhi and reported *Aphis gossypii* Glov. to be the vector. During the year 1956–57 investigations were undertaken with a view to determine if there are any other aphids besides *Aphis gossypii* which may transmit the chilli mosaic virus, and also to study their relative efficacy to transmit the virus to healthy chilli plants.

Three additional species of aphids were collected from different crops such as *Myzus persicae* Sulz. from potato, *Lipaphis erysimi* Kalt from cabbage and *Aphis evonymi* Fabr. from *Solanum nigrum*, and colonised in the insectary on their respective host plants except *Myzus persicae* which was colonised on tobacco. Large number of adult, apterous forms of all the aphid species, including *Aphis gossypii* were taken in separate Petri plates by means of a camel hair brush and starved for one hour. They were then allowed to feed on mosaic affected chilli plants for five minutes. The aphids were removed to Petri plates after this period and groups of ten aphids were transferred to healthy test chilli plants for inoculation feeding. After 24 hours, the aphids were removed and plants sprayed with 0.1 percent Ekatox solution. The experiment was repeated thrice with ten plants inoculated with each species of aphid. The results of such tests are summarised below:

Aphid species	Number of plants infected in each trial (out of 10)			Total No. of plants infected (out of 30)	Percentage of infection
	I 20.2.57	II 1.3.57	III 10.3.57		
Aphis gossypii ...	6	8	8	22	73.3
Myzus persicae ...	5	8	6	19	63.3
Aphis evonymi ...	0	2	1	3	10.0
Lipaphis erysimi ...	0	0	0	0	00.0

*Originals not seen.

In addition to *Aphis gossypii*, the chilli mosaic virus was transmitted by *Myzus persicae* and *Aphis evonymi* but it could not be transmitted by *Lipaphis erysimi*. *Aphis gossypii* and *Myzus persicae* were found to be almost equally efficient whereas transmission efficiency of *Aphis evonymi* was much lower.

Grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology for his helpful suggestions and keen interest. Thanks are also due to Dr. E. S. Narayanan, Head of the Division of Entomology for kindly identifying the insect species.

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Certain Morphological Peculiarities in races 34 and 75 of Puccinia graminis tritici (Pers.) Erikss. & Henn.—L. M. Joshi and Dulari Kak. The concept of physiologic races arose when it was shown that the *Forma specialis* could be further sub-divided into different forms. Stakman and Lavine (1922) tested a large number of varieties against different isolates of *P. graminis tritici* and selected 12 varieties known as differential hosts, for isolating different forms or races, which though morphologically alike are distinct from one another in their capacity to infect differential hosts. The idea of morphological similarity does not, however, always hold good for all the races. Many workers have observed slight differences amongst physiologic races. It has been recorded by Johnston (1930) that race 27 differs from all other common races in the pustule size, colour, thickening of spore walls, irregularity of uredospore and also the size of the uredospores. Chester (1946) too has recorded that race 21 had an incubation period longer than that of other races.

Some similar though consistent differences have been observed amongst a few Indian races of black rust of wheat. The chief morphological characteristics of the two races are given below:—

Race 34: This race was first isolated in India in 1940 and a single spore culture of the race has been maintained at Mycological Substation, Simla (7,000 ft. a.s.l.) since then. The colour (Ridgway-1912) of uredo-pustule

is Sudan Brown sometimes changing to Amber Brown whereas other races are usually Chestnut or Argus Brown in colour gradually changing with age.

Another peculiarity with this race is the size of the uredospores. The uredospores of this race are smaller in length by 3–4μ than the uredospores of other races. The average measurement of 100 uredospores (12 days in age) being 14.4 to 25 μ by 4.7 to 8 μ. (with a range of 18.8 to 34.5 μ by 12.5 to 18.8 μ) as against the average measurement of 15 μ to 28.5 μ by 4.8 to 8.5 μ (with a range of 18.9 to 36.5 μ by 12.5 to 19.0μ).

Race 75. Though this race was one of the first recorded races in India, it has practically ceased to exist as it has not been isolated since 1938. This race differs from all other races picked up so far in this country in its incubation period which is more than one and half times longer than that of other races. It has been observed that at a temperature range of 57°F to 76°F the usual incubation period of all the races is 7 to 8 days whereas under identical conditions the pustules of race 75 do not appear earlier than 11 or 12 days. Another peculiarity with this race is the non-erumpent pustules. It has been observed that in most races epidermis ruptures within a day or two after incubation period but with race 75, at times, the epidermis does not rupture even after the seedling leaves wither away. In winter months particularly under conditions of high humidity, quite often, the epidermis does not rupture at all. Under normal circumstances, when environmental conditions are most congenial, the epidermis ruptures only very slightly, that too when cultures have become pretty old, with the result that very little uredo-dust can be collected unless the epidermis is artificially removed. This persistent epidermis, however, is by no means restricted alone to Agra local wheat (an indigenous susceptible variety of *Triticum vnlgare*, used for maintenance of rust cultures) but has been observed in some other varieties too. It might also be mentioned here that some other races like 15–C also exhibit this character, but not to such a marked extent.

It is, however, obvious that separation of races on the basis of the above morphological characters is not possible. Some of the characters described above are interesting and may, to some extent, account for the spread and prevalence of the race. For instance, a race with a longer incubation period will not have the same chance of spread in the field, as other races with shorter incubation period. This is particularly so for a race like 75 which has so far been comparatively a rare race. Another factor that might have contributed to the restricted distribution of the race, is perhaps the persistent epidermis which does not permit easy release of the uredospores. Such factors would appear to be significant in the spread of a race particularly in the plains of India where black rust appears for less than 3 months. Also success or failure of a physiologic race in nature would depend on the combined effect of a number of ecological factors operating together, however insignificant they may appear to be.

Acknowledgement. Our grateful thanks are due to Dr. R. S.

Vasudeva, Head of the Division and Dr. R. Prasada for their guidance and helpful suggestions.

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Authors are invited to consult Bisby's 'An Introduction to Taxonomy and Nomenclature of Fungi' (1945), p.p. 38-41 and Riker's "The preparation of manuscripts for *Phytopathology*, *Phytopathology* **36** : 953-977, 1946, before preparing their mss. and figures.

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Published & Edited by R. Prasada for the Indian Phytopathological Society
Pusa Institute, New Delhi-12
and Printed at Oxford Printing Works, New Delhi.